GROWTH RESPONSE OF TROPICAL FORAGE LEGUMES TO INOCULATION WITH VA MYCORRHIZAL FUNGI AND PHOSPHORUS APPLICATION

By

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
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To the memory of my father Efrain, who influenced me in a very special way. I am very heartbroken that he died before this work was completed.

To my mother, Guillermina, for her never ending sacrifices, her love, and prayers.

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December 1987

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Greenhouse and field studies were conducted to determine the growth response of tropical forage legumes to inoculation with vesicular-arbuscular mycorrhizal (VAM) fungi and P applications and to evaluate the effectiveness of the indigenous VAM population versus introduced species.

Root and rhizosphere soil samples of four tropical forage legume species were collected at four locations in south Florida before initiating the greenhouse experiments. Six species of VAM fungi were isolated in this survey. The occurrrence of VAM fungal species, as determined by spore numbers, was affected by legume species and location.

Shoot dry and root dry weights of 'Siratro' (Macroptilium atropurpureum Urb.), aeschynomene (Aeschynomene americana L.),

Aeschynomene villosa Poir., Stylo (Stylosanthes guianensis SW.), and Stylosanthes hamata Taub. were increased in pasteurized and nonpasteurized limed soil in the greenhouse after inoculation with Glomus intraradices Schenck & Smith.

Inoculation with Glomus etunicatum Becker & Gerdemann and G. intraradices also increased the growth of Siratro as compared to other VAM fungi and the noninoculated control in limed, nonpasteurized soil fertilized with 20 mg kg^{-1} of P. In other greenhouse experiments, G. etunicatum and G. intraradices were effective growth enhancers of Siratro over a practical range of 2.5 to 40 mg kg^{-1} of applied P in a limed, nonpasteurized soil. For both fungi, increasing P above 2.5 mg kg^{-1} increased the percentage and total root length colonized by VAM fungi. A positive correlation was found between mycorrhizal root colonization and shoot dry weight. In a limed, pasteurized soil, inoculation with G. etunicatum increased total P and N of Siratro at 12.5 and 25 mg kg^{-1} of applied P, but not at 50 mg kg^{-1}.

The effectiveness of G. etunicatum and G. intraradices with Siratro and aeschynomene was corroborated in a field trial. These fungi increased the growth and uptake of P and N of both legumes over a range of applied P from 10 to 80 kg ha^{-1}.

Inoculation of forage legumes with effective VAM fungi enhanced their growth. Growth enhancement occurred at P and lime levels used in commercial pasture production and in soils that had a large, but apparently ineffective indigenous VAM population.

CHAPTER I
INTRODUCTION

Forage legumes are an important component of improved grass pastures and must be established rapidly and without excessive cost. The legumes serve both to increase forage quality and decrease the need for N fertilizer through N_2-fixation.

Newly cleared lands incorporated into pasture production in south Florida are generally acidic and very low in total and available P throughout the soil profiles. While improvements to the productivity of these pastures may be obtained by the introduction of suitable legumes, effective N_2-fixation and establishment of legumes is frequently limited by the low levels of available P in these soils. Snyder et al. (1978) reported that large applications of P fertilizer are normally required for legume establishment and optimun growth in these soils. However, with the increasing cost of P fertilizer, alternative strategies for minimum P fertilizer input and efficient use of P must be adopted. One of these strategies may be via the management of vesicular-arbuscular mycorrhizal (VAM) symbioses.

Vesicular-arbuscular mycorrhizal fungi can improve the growth of legumes by increasing P uptake (Bethlenfalvay et al., 1985; Hayman, 1983). Phosphorus is often a growth-limiting factor since many legumes have P requirements and are poor scavengers of P. The VAM fungi may also increase nodulation and N_2-fixation of legumes, primarily as an indirect

effect of improved P nutrition (Daft and El-Giahmi, 1976; Habte and Aziz, 1985).

Information concerning the association of VAM fungi with tropical forage legumes is sparse. Most of the growth response studies reported were done in either pasteurized soil or in small volumes of nonpasteurized soil. Except for the work of Saif (1987), little information is available on growth response of tropical forage legumes to inoculation with VAM fungi in nonpasteurized soil, especially under field conditions where introduced species of VAM fungi must compete with the indigenous VAM population.

Therefore, greenhouse studies were conducted in limed, pasteurized and nonpasteurized soil to improve the growth of tropical forage legumes through inoculation with effective VAM fungi and reduced P fertilization. In addition, the effect of inoculation with selected VAM isolates on growth and nutrient uptake of two tropical forage legumes under natural field conditions was investigated at different levels of applied P.

CHAPTER II
THE OCCURRENCE OF VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGI ON TROPICAL FORAGE LEGUMES IN SOUTH FLORIDA.

Introduction

There is widespread interest in the use of tropical forage legumes to increase production of tropical grasses in Florida's beef-cattle industry (Snyder et al., 1985). These legumes respond to inoculation with VAM fungi (Lynd et al., 1985; Saif, 1987)). However, before initiating fungal inoculation experiments with these forage legumes in south Florida, a survey was needed of the native populations of VAM fungi associated with several commercial forage legumes growing on a variety of soils.

Vesicular-arbuscular mycorrhizal associations have been observed in a wide variety of natural and agricultural ecosystems (Abbott and Robson, 1977a; Currah and Van Dyk, 1986; Harley and Harley, 1987). In Florida, the occurrence and distribution of VAM fungi in agronomic crops, including some tropical legumes (Schenck and Kinloch, 1980; Schenck and Smith, 1981), and sand-dune vegetation (Sylvia, 1986), has been reported. However, there is no information on the degree of native VAM colonization of tropical forage legumes in Florida or on the susceptibility of different species of legumes to various genera and species of VAM fungi.

The objective of this survey was to obtain quantitative data on the amount of root colonization and the species distribution of VAM fungi

associated with four cultivated tropical forage legumes from four different locations in south Florida.

Materials and Methods

Root and rhizosphere soil samples of four tropical forage legumes were collected from 11 to 17 October 1984, at four locations in south Florida: Deseret Ranches, Deer park; Fort Pierce, Agricultural Research and Education Center (AREC); Ona, AREC; and Basinger Ranch, 109 Ranch (Fig. 2-1). Most of the soils of the studied area belong to the order Spodosols. They are dominated by nearly level, somewhat poorly to poorly drained sandy soils with dark sandy subsoil layers. These soils are used primarily for pastures, vegetables, flowers, forestry, and citrus.

The forage legumes sampled were: 'Siratro' (*Macroptilium atropurpureum* Urb.), (except at Deseret Ranches), aeschynomene (*Aeschynomene americana* L.), *Vigna adenantha* Marechal, Mascherpa and Stainier, and carpon desmodium (*Desmodium heterocarpon* DC.). The legumes were mixed with pasture grasses at the time of sampling. Three rhizosphere soil samples were collected to a depth of 15 cm for each legume at each location. Samples, consisting of three subsamples of approximately 1.5 kg, were placed in plastic bags and transported to the laboratory on the same day.

Samples were sieved through a 4-mm screen, and 100 g subsamples were removed and stored at $5^{o}C$ for spore extraction. Legume roots were carefully separated manually from grass roots. A portion (0.5 g) of each

Fig. 2-1. Collection sites for VAM fungi associated with four tropical forage legumes in south Florida.

root sample was cleared in 10% KOH and stained with 0.05% trypan blue in lactophenol (Kormanik and McGraw, 1982). Root colonization by VAM fungi was estimated by the gridline-intersect method of Giovannetti and Mosse (1980).

Chemical content of a composite soil sample from each location was determined by the Soil Testing Laboratory, University of Florida (Rhue and Kidder, 1984). Mehlich-I solution (0.05 M HCl + 0.0125 M H_2SO_4) was used to extract Al, Ca, K, Mg, and P. All elements were analyzed in the filtrate by atomic absorption spectrophotometry, except P which was determined using the ammonium molybdate/ascorbic acid colorimetric method. Soil pH was determined using a 1:2 (v/v) soil:water ratio. Organic matter was estimated by oxidation with 1 N $K_2Cr_2O_7$ in the presence of H_2SO_4.

Spores of VAM fungi were removed from soil by the wet sieving method of Daniels and Skipper (1982) using sieves with 425, 90, and 45 um openings. Fractions retained on 90 and 45 um sieves were centrifuged (1000 x g) for 3 min in water. The pellet was resuspended in 40% sucrose solution and centrifuged for 1.5 min. Spore species were identified where possible (Schenck and Smith, 1982; Trappe, 1982). In addition, spores or washed roots were placed in pasteurized Arredondo loamy sand surface soil (siliceous hyperthermic Grossarenic Paleudult) in 15-cm-diam plastic pots in the greenhouse and planted with bahiagrass (*Paspalum notatum* Flugge), carpon desmodium, or Siratro in an attempt to isolate VAM fungi in a manner similar to that described by Gerdemann and Trappe (1974) as the "inoculated pot culture" method.

Results and Discussion

Results of soil pH and chemical analysis of soil samples reflect the different management regimes (including lime and fertilizer) (Table 2-1).

Differences in percentage of mycorrhizal root colonization and total spore density of air dry soil were significant among locations, legume species, and location x legume species interactions (Table 2-2). Total spore density at the four locations ranged from 5 to 679 per 100 g of air-dried soil, and the percentage of mycorrhizal root colonization varied from 3 to 41%. Miller et al. (1979) observed variable degree of mycorrhizal root colonization (4 to 74%) in forage grasses and legumes in Brazil. Except for carpon desmodium, legume species differed in percentage root colonization and total spore density among locations (Table 2-3). Fort Pierce had the highest total spore density for each legume species sampled except for Siratro.

Attempts were made to relate percentage root colonization and total spore density to soil P or the other soil chemical characteristics presented in Table 2-1, but no clear relationships were apparent. Abbott and Robson (1977a) and Hayman (1978) also reported that spore numbers were not correlated with soil P or soil pH in cultivated soils.

There was a positive correlation ($P < 0.05$) between root colonization and total spore density for all legume species at Basinger ($r = 0.70$) and Deseret ($r = 0.76$), but not at Fort Pierce and Ona. Giovannetti (1985) and Miller et al. (1979) reported a correlation

Table 2-1. Chemical characteristics of the soils sampled for VAM fungi associated with four tropical forage legumes at four locations in south Florida.

Location	Legume species[z]	O.M.	pH	Al	Ca	Mg	K	P
		%		---------mg kg^{-1} soil----------				
Fort Pierce	AA	1.4	6.0	23	314	93	8	4
	DH	1.2	5.3	22	241	15	16	5
	VA	1.3	5.5	25	242	21	20	4
	MA	1.3	5.2	62	270	25	13	16
Ona	AA	3.4	6.1	44	1320	143	64	23
	DH	3.1	5.4	36	920	120	29	8
	VA	2.5	4.9	22	480	70	46	8
	MA	5.7	4.7	55	800	95	43	5
Deseret	AA	2.5	6.1	66	780	67	40	6
	DH	2.3	6.0	27	1040	94	27	4
	VA	2.8	7.2	30	1600	141	55	9
Basinger	AA	4.7	5.3	28	960	32	28	4
	DH	3.5	5.2	26	460	100	46	7
	VA	3.6	5.1	28	540	111	58	8
	MA	4.2	5.2	36	640	129	94	11

[z]AA= *Aeschynomene americana*; DH= *Desmodium heterocarpon*; VA= *Vigna adenantha*; MA= *Macroptilium atropurpureum*.

Table 2-2. Analysis of variance for percentage of mycorrhizal root colonization and total spore density per 100 g of air-dried soil.

Source of variation	Degree of freedom	Mean Squares: Root colonization	Mean Squares: total spore density
		%	no. 100 g^{-1} soil
Location	3	447	325871
Legumes	3	321	73895
Interaction	8	299**	88933**
Error	30	13	1320

**Significant at $P < 0.01$

Table 2-3. Mean percentage of mycorrhizal root colonization and total spore density of VAM fungi among forage legumes at four locations in south Florida.

Location	Root colonization[z]	Total spore density[z]
	%	no. 100 g^{-1} soil
	Aeschynomene americana	
Ft. Pierce	7[b]	302[a]
Ona	6[b]	160[b]
Basinger	5[b]	8[c]
Deseret	30[a]	146[b]
	Desmodium heterocarpon	
Ft. Pierce	12[a]	679[a]
Ona	15[a]	376[b]
Basinger	12[a]	19[c]
Deseret	16[a]	36[c]
	Vigna adenantha	
Ft. Pierce	5[c]	535[a]
Ona	41[a]	77[b]
Basinger	20[b]	25[c]
Deseret	25[b]	36[c]
	Macroptilium atropurpureum	
Ft. Pierce	15[a]	23[b]
Ona	8[b]	294[a]
Basinger	3[b]	5[b]

[z]Means within a column for each legume species followed by the same letter are not different ($P < 0.05$) according to Duncan's multiple range test.

between root colonization and spore density, while Hayman and Stovold (1979) and Giovannetti and Nicolson (1983) found no correlation. This apparent discrepancy may be due to different sampling methods. Giovannetti (1985) collected samples within the same plant species and sites, while the other researchers collected samples from many different plant species and sites.

Spore production and root colonization are influenced by seasonal variations (Giovannetti, 1985; Sylvia, 1986), host plant, stage of development (Saif and Khan, 1975; Schenck and Kinloch, 1980), and soil type (Lopes et al., 1983). In this survey there was only one sampling, so it was not possible to separate seasonal or host developmental effects on root colonization and total spore density.

The 6 species of VAM fungi collected in this survey were: Gigaspora heterogama (GH) Gerdemann & Trappe, Gigaspora margarita (GM) Becker & Hall, Glomus etunicatum (ETU) Becker & Gerdemann, Glomus intraradices (INT) Schenck & Smith, Glomus sp. (GS), and Acaulospora spinosa (AS) Walker & Trappe. The unidentified Glomus sp. was dark brown to black, 200-250 µm in diam, and had 1 wall of 8-14 µm thickness.

The occurrence of fungal species, as determined by spore numbers, was affected by the legume host and location (Table 2-4). Iabal et al. (1975) and Schenck and Kinloch (1980) also recorded differences in spore numbers among plant species. The maximum number of spores of G. margarita occurred at Fort Pierce associated with aeschynomene. Spores of G. margarita were not found associated with Siratro at any of the four locations. Spores of G. heterogama, G. etunicatum, and G. intraradices

Table 2-4. Mean spore numbers of VAM fungal species associated with four forage legumes at four locations in south Florida.

Location	Species of VAM fungi[z]					
	GM	GH	ETU	INT	AS	GS
			Aeschynomene americana			
Ft. Pierce	141[a]	66[b]	21[a]	42[b]	0	16
Deseret	9[b]	136[a]	0	0	0	0
Basinger	0	8[c]	0	0	0	0
Ona	0	16[c]	29[a]	114[a]	0	0
			Desmodium heterocarpon			
Ft. Pierce	2[a]	114[a]	255[b]	307[a]	0	0
Deseret	2[a]	9[b]	0	0	0	0
Basinger	0	0	19[c]	0	0	0
Ona	0	0	325[a]	51[b]	0	0
			Macroptilium atropurpureum			
Ft. Pierce	0	10[a]	0	4[b]	0	8
Basinger	0	5[b]	0	0	0	0
Ona	0	0	40	254[a]	0	0
			Vigna adenantha			
Ft. Pierce	1[b]	39[a]	252[a]	241[a]	0	0
Deseret	20[a]	15[b]	0	0	0	0
Basinger	0	0	25[b]	0	0	0
Ona	28[a]	0	28[b]	11[b]	9	0

[z]Means within a column for each legume species followed by the same letter are not different ($P < 0.05$) accordingly to Duncan's multiple range test.

were found associated with all legumes, in at least one of the locations. Glomus heterogama occurred in greatest numbers at Deseret and Fort Pierce associated with aeschynomene and carpon desmodium, respectively. The maximum number of spores of G. etunicatum occurred at Ona and Fort Pierce associated with carpon desmodium. A high number of spores of G. etunicatum was also found at Fort Pierce associated with Vigna adenantha. Glomus intraradices was recovered in greater numbers from carpon desmodium and Vigna adenantha at Fort Pierce as well as from Siratro at Ona. The unidentified Glomus sp. occurred in lower numbers than the other two species of Glomus; it was only found at Fort Pierce, associated with aeschynomene and Siratro. Acaulospora spinosa was only recovered from Vigna adenantha at Ona.

Overall root colonization by VAM fungi was low (most values below 20%) which indicates that (1) the native population of VAM fungi is not very infective and (2) field inoculation may be effective. Attempts to establish pot cultures of VAM fungi recovered in this survey were only successful with G. etunicatum and G. intraradices. These two fungi were shown to be effective in increasing the growth of several forage legumes and were chosen for further evaluations.

CHAPTER III
GROWTH RESPONSE OF TROPICAL FORAGE LEGUMES TO INOCULATION WITH GLOMUS INTRARADICES

Introduction

The use of forage legumes as companion crops to increase production of grasses is becoming an established practice in order to reduce the requirement for N fertilization (Rotar, 1983). In soils where P is a limiting factor, large applications of P fertilizer are required for legume establishment and optimum growth. However, with the increasing cost of P fertilizer, alternative strategies for minimum input and efficient use of P must be adopted. It is pertinent, therefore, to evaluate whether mycorrhizal associations with forage legumes can be manipulated in order to improve establishment, P nutrition, N_2-fixation, and consequently yield.

Vesicular-arbuscular mycorrhizal fungi can improve the growth of legumes by increasing P uptake (Bethlenfalvay et al., 1985; Chulan and Ragin, 1986; Harley and Smith, 1983; Hayman, 1983; Jensen, 1984). Phosphorus is often a growth-limiting factor since many legumes have high P requirements and are poor scavengers of P. The VAM fungi may also increase nodulation and N_2-fixation of legumes, primarily as an indirect effect of improved P nutrition (Daft and El-Giahmi, 1976; Habte and Aziz, 1985; Newbould and Rangeley, 1984).

Most of the literature concerning the association of VAM fungi with forage legumes is on temperate species; e.g. alfalfa (Medicago sativa L.) (Kucey and Diab, 1984; Nielsen and Jensen, 1983; Satterlee et al., 1983), white clover (Trifolium repens L.) (Newbould and Rangeley, 1984; Powell, 1979; Rangeley et al., 1982), and subterranean clover (Trifolium subterraneum L.) (Abbott and Robson, 1978). Studies on tropical forage legumes have been limited to a few species such as tropical kudzu (Pueraria phaseoloides Benth) (Salinas et al., 1985; Waidyanatha et al., 1979), leucaena (Leucaena leucocephala Dewit) (Huang et al., 1985) and Stylo (Stylosanthes guianensis SW.) (Mosse, 1977; Waidyanatha et al., 1979).

The purpose of this investigation was to evaluate the effect of a VAM fungus, G. intraradices, on the growth of several tropical forage legumes in pasteurized and nonpasteurized soil under greenhouse conditions.

Materials and Methods

The top 15 cm of a virgin Oldsmar fine sand (sandy, siliceous, hyperthermic Alfic Haplaquods) was collected from a newly cleared area at the Agricultural Research and Educational Center, Fort Pierce, FL. The low-P soil was air-dried and sieved through a 4-mm screen. The soil had an initial pH of 4.5 (1:2 soil:water suspension) and P, Ca, Mg, and K concentrations (extracted with 0.05 M HCl + 0.0125 M H_2SO_4) of 1, 63, 21 and 12 mg kg^{-1} soil, respectively. Lime, as high calcitic limestone, was thoroughly incorporated at a rate of 1500 mg kg^{-1} soil (equivalent

to 3,000 kg ha^{-1} assuming a 15-cm depth of soil ha^{-1} with a bulk density of 1.3 g cm^{-3}) and allowed to equilibrate for 30 days before initiating the study. Solutions of P, K, Mg, Cu, Mn, Zn, B, and Mo also were thoroughly mixed with the soil to supply rates of 10, 30, 12, 1.5, 1.0, 1.0, 0.50 and 0.10 mg kg^{-1}, respectively. A portion of the soil was pasteurized at 60°C for 4 h to eliminate the indigenous mycorrhizal fungi. Then 3 kg of soil was placed into 15-cm-diam plastic pots. The pH of the soil was 6.2 at the end of the experiment.

The legumes used in the experiment were: Siratro, aeschynomene, *Aeschynomene villosa* Poir., Stylo, leucaena, *Stylosanthes hamata* Taub., cv. 'Verano', *Vigna adenantha*, and *Arachis* sp. Seeds were scarified with sandpaper, wetted, and sprinkled with type EL "cowpea" inoculum (Nitragin Co., Milwaukee, WI) prior to planting. Five seeds of the corresponding legumes were planted per pot, and plants were thinned to one per pot 10 d after emergence.

Glomus intraradices (isolate S311), used in this study, was isolated from cultivated *Vigna adenantha* at the Agricultural Research and Education Center, Ona, FL. (Chapter II, Table 2-4). Fungal inoculum was produced in pot culture in pasteurized soil containing carpon desmodium as the host plant. Pot cultures were 10-weeks old when they were harvested, mixed and used to inoculate experimental pots. Ten grams per pot of the soil-root-fungus inoculum containing approximately 200 spores was spread in a 1-cm-thick layer, at a depth of 3 to 5 cm below the soil surface. Noninoculated control treatments received 10 g of a soil-root mixture from noninoculated pot cultures that were free of VAM fungi.

The experimental treatments consisted of pasteurized or nonpasteurized soil, with or without addition of _G. intraradices_ inoculum. The pots were arranged on greenhouse benches in a completely randomized design with three replications per treatment. The average maximum and minimum greenhouse temperatures were 37 and 26°C, respectively. Pots were watered as needed to maintain soil moisture near field capacity and were re-randomized every two weeks.

Plants were harvested after 45 d. Shoot and roots were dried at 70°C for 48 h and weighed. The percentage of mycorrhizal root colonization was determined as described in Chapter II.

Significant treatment effects on shoot and root dry weights within legume species were analyzed by the T TEST procedure of the Statistical Analysis Systems (SAS Institute, 1982).

Results and Discussion

Inoculation with _G. intraradices_ in nonpasteurized soil resulted in greater shoot dry weights ($P < 0.05$) for five of the seven legumes tested (Fig. 3-1). Greater shoot dry weights of these legumes were positively related to increased levels of mycorrhizal colonization following inoculation (Table 3-1).

Root dry weight results were similar to those for shoot dry weight, except for Stylo, where there was no increase in root dry weight as a result of mycorrhizal inoculation (Fig. 3-1).

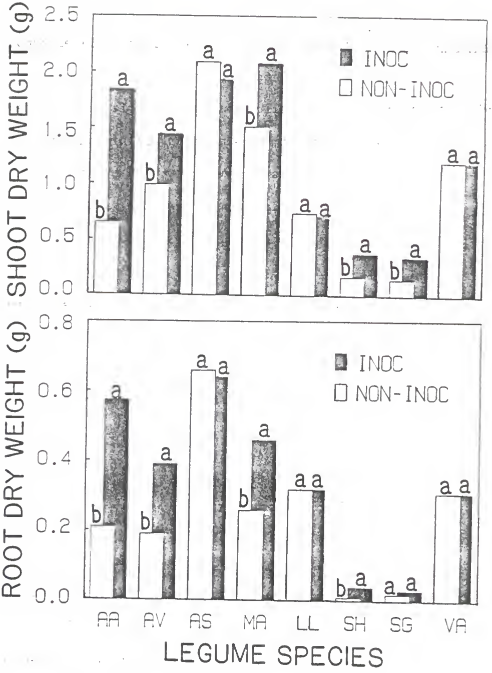

Fig. 3-1. Effect of inoculation with Glomus intraradices on the shoot and root dry weights of tropical forage legumes in nonpasteurized soil in the greenhouse after 45 days. Legume species were Aeschynomene americana (AA), Aeschynomene villosa (AV), Arachis sp. (AS), Macroptilium atropurpureum (MA), Leucaena leucoephala (LL), Stylosanthes hamata (SH), Stylosanthes guianensis (SG), and Vigna adenantha (VA). Bars represent the mean of 3 replicates. Means with the same letter within a species are not different ($P < 0.05$).

Table 3-1. Percentage of mycorrhizal root colonization of tropical forage legumes in nonpasteurized (UP) or pasteurized (P) soil in the greenhouse after 45 days.

Legume species	Mycorrhizal inoculation	Root colonization[z] UP	P
		----- % -----	
Aeschynomene americana	+	22	3
	-	5	0
Macroptilium atropurpureum	+	49	35
	-	20	0
Aeschynomene villosa	+	10	*
	-	6	
Stylosanthes hamata	+	12	*
	-	6	
Stylosanthes guianensis	+	28	*
	-	8	
Vigna adenantha	+	59	53
	-	40	0
Leucaena leucocephala	+	4	3
	-	1	0
Arachis sp.	+	31	12
	-	35	2

[z]Based on a composite of three samples for each legume.

*Treatment lost to glasshouse accident.

There was no increase in plant growth as a result of inoculation with G. intraradices in nonpasteurized soil for Arachis sp., leucaena, and Vigna adenantha. With the exception of leucaena, this may be attributed to effective colonization by the indigenous mycorrhizal fungi in the noninoculated soil (Table 3-1).

Only five legume species (Fig. 3-2) were evaluated in pasteurized soil; three were lost in a greenhouse accident. Siratro, Arachis sp., and Vigna adenantha had greater shoot and root dry weights after G. intraradices inoculation. This increase again was related to effective root colonization by G. intraradices (Table 3-1). In another study, Siratro was shown to respond to inoculation with several VAM fungi in pasteurized soil in the greenhouse (Lopes and De Olivera, 1980).

Inoculation with G. intraradices did not increase either the shoot or root dry weights of aeschynomene or leucaena in pasteurized soil. However, mycorrhizal colonization on both legumes was very low (3%).

Leucaena has been reported to be very mycorrrhizal dependent because it has few root hairs (Huang et al., 1985; Yost and Fox, 1979). The failure of VAM fungi to colonize it in this study in both pasteurized and nonpasteurized soil may be due to incompatibility between the plant and G. intraradices , as well as native VAM fungi in the experimental soil, to inhibitory soil factors on the host-VAM symbiosis, or to the relatively slow development of the root system. It has been shown that some mycorrhizal fungi may be less effective on certain plant hosts. For example, Schroder et al. (1977) reported that Glomus macrocarpum Tul & Tul increased growth of onions but not of Stylosanthes sp.

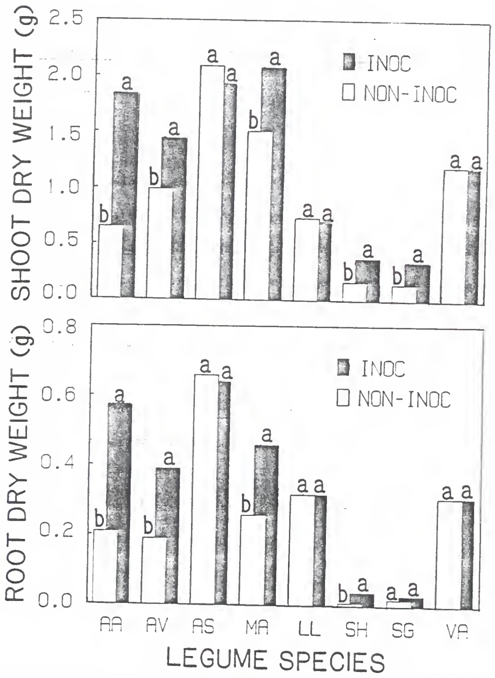

Fig. 3-2. Effect of inoculation with Glomus intraradices on the shoot and root dry weights of tropical forage legumes in pasteurized soil in the greenhouse after 45 days. The legume species were Aeschynomene americana (AA), Arachis sp. (AS), Macroptilium atropurpureum (MA), Leucaena leucocephala (LL), and Vigna adenantha (VA). Bars represent the mean of 3 replications. Means with the same letter within a species are not different ($P < 0.05$).

The failure to obtain good colonization of aeschynomene in pasteurized soil was unexpected, since this legume was successfully colonized in nonpasteurized soil. Unfortunately, soil chemical properties were not determined after pasteurization. Legumes are sensitive (and hence VAM fungi) to elevated Mn, which is a common occurrence in heat treated soils.

The results obtained in this study clearly demonstrate that *G. intraradices* can successfully compete with some of the indigenous mycorrhizal fungi present in the experimental soil and promote growth of several legumes in nonpasteurized soil. This result agrees with earlier work by Abbott and Robson (1981), Mosse (1977), and Rangeley et al. (1982), which suggests a potential for successful field-scale inoculation with effective VAM fungi.

CHAPTER IV

GROWTH RESPONSE OF SIRATRO TO INOCULATION WITH VA MYCORRHIZAL FUNGI IN NONPASTEURIZED SOIL. I. SELECTION OF EFFECTIVE VA MYCORRHIZAL FUNGI UNDER AMENDED SOIL CONDITIONS.

Introduction

Siratro, a cultivar developed by E. M. Hutton (1962) from two Mexican accessions of *Macroptilium atropurpureum* Urb., is a persistent, perennial forage legume adaptable to a wide range of soil and climatic conditions. It has become widespread and is among the most versatile forage legume grown throughout tropical regions of the world (Lynd et al., 1985). In pasteurized and nonpasteurized soils, increased growth of Siratro was attained after inoculation with *Glomus fasciculatum* Gerdemann & Trappe (Lopes and De Olivera, 1980; Lynd et al., 1985) and *G. intraradices* (Chapter III).

Hayman (1982) stated that VAM fungi are probably capable of symbiosis with most plants, at least to some degree. However, there is wide variation in the ability of VAM fungi to stimulate plant growth (Miller et al., 1985; Powell, 1982; Schubert and Hayman, 1986). Lopes and De Olivera (1980), using a gamma-irradiated soil of low P-content, studied the effect of inoculation with nine species of VAM fungi on the growth of Siratro. Only inoculation with *G. fasciculatum* and *G. macrocarpum* enhanced plant growth. Abbott and Robson (1981) defined the relative ability of a VAM fungus to stimulate plant growth as 'effectiveness' and

this defined term will be used in this paper. Wilson (1984) indicated that an evaluation of the effectiveness of the indigenous mycorrhizal population under amended soil conditions, as well as studies to select effective VAM fungi, are prerequisites for successful field inoculation.

Thus, the objective of the present study was to determine the effectiveness of several VAM fungi with Siratro in a limed, nonpasteurized soil with low P content under greenhouse conditions.

Materials and Methods

The soil used in this study, liming, and fertilizer amendments are described previously in Chapter III.

Plants were inoculated with the following VAM fungi: *G. etunicatum* (isolate S312) obtained from carpon desmodium at the Agricultural Research and Education Center, Ona, FL. (Chapter II, Table 2-4); *G. deserticola* Trappe, Bloss & Menge (isolate S305) obtained from sea oats (*Uniola paniculata* L.) in a coastal dune, Anastasia, FL.; *G. versiforme* Berch & Fortin (isolate #231) obtained from N.C. Schenck, University of Florida, Gainesville, FL.; *G. intraradices* (isolate S311) obtained from *Vigna adenantha* at the Agricultural Research and Education Center, Ona, FL. (Chapter II, Table 2-4); *G. margarita* (isolate #215) obtained from N.C. Schenck, University of Florida, Gainesville, FL. Isolates were maintained in pot cultures in pasteurized soil containing bahiagrass. Soils from 12-week-old pot cultures were used to inoculate experimental pots. The propagule densities of the native soil and inocula at the beginning of the experiment were determined by the most-probable-number

(MPN) technique using bahiagrass as the host plant and pasteurized Oldsmar fine sand as the diluent (Daniels and Skipper, 1982). The amount of inoculum used was adjusted to give equal inoculum densities among isolates. Each pot received approximately 240 propagules. Details on the fungal inoculation technique, planting, and watering were reported previously (Chapter III).

There were six inoculation treatments, five species of VAM fungi, and a control inoculated with non-VAM pot culture material. The pots were arranged on the greenhouse bench in a completely randomized block design with 15 replications per treatment.

The average maximum and minimum greenhouse temperatures during the experimental period were 32 and 19°C, respectively. Maximum photosynthetic photon flux density was 1200 μ mol m^{-2} s^{-1}.

Five randomly selected samples were harvested from each treatment after 20, 40, and 70 d of growth. At first harvest, shoot dry weight, percentage of root colonized by VAM fungi, plant height, and number of leaves were determined. In addition, root fresh weight and total root length colonized were determined at the second and third harvests. Shoot dry weight was determined by drying the material at 70°C for 24 h. Percentage and total root length colonized were estimated by the gridline intersect method (Giovannetti and Mosse, 1980) after roots were cleared in 10% KOH and stained with 0.05% trypan blue in lactophenol (Kormanik and McGraw, 1982). Data were analyzed by Analysis of Variance Procedure, Statistical Analysis Systems (SAS Institute Inc., 1982). Duncan's multiple range test was used to separate treatment means when the F-test was significant ($P < 0.05$).

Results and Discussion

Plants inoculated with *G. etunicatum* and *G. intraradices* had higher shoot dry and root fresh weights than plants inoculated with the other VAM fungi or control plants, at 40 and 70 d after planting (Fig. 4-1). At both harvests, plants inoculated with *G. etunicatum* had higher shoot dry weights than plants inoculated with *G. intraradices*. At the final harvest, plants inoculated with *G. intraradices* had higher root fresh weights than plants inoculated with *G. etunicatum*.

In contrast, plants inoculated with *G. versiforme*, *G. margarita*, and *G. deserticola* had shoot dry and root fresh weights that were not different from the noninoculated plants, except at the final harvest when plants inoculated with *G. deserticola* had higher shoot dry and root fresh weights than the control (Fig. 4-1). At 20 d, shoot dry weights were not different among treatments (mean = 0.70 g).

Percentage and total root length colonized by VAM fungi increased with time (Fig. 4-2). Inoculation with *G. etunicatum* and *G. intraradices* resulted in the highest root colonization at all harvests. At 70 d, plants inoculated with *G. etunicatum* had the highest root colonization, followed by *G. intraradices* and then *G. deserticola*. There were no differences in root colonization among *G. versiforme*, *G. margarita* and control treatments. For the six treatments, total root length colonized by VAM fungi and percentage of mycorrhizal root colonization followed the same trend (Fig. 4-2).

Shoot dry weight of Siratro was correlated with total root length colonized by VAM fungi ($r^2 = 0.95^{**}$) and percentage of mycorrhizal root

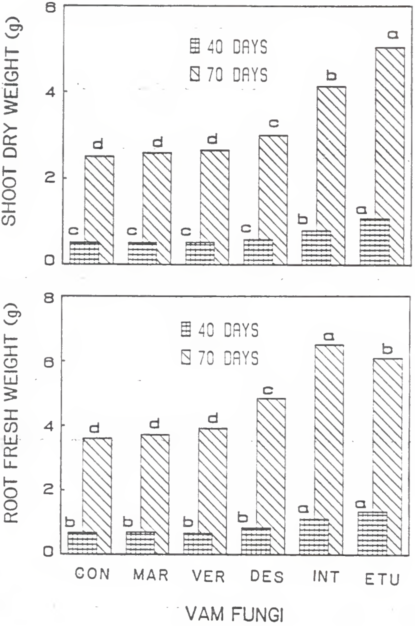

Fig. 4-1. Effect of inoculation with Gigaspora margarita (MAR), Glomus versiforme (VER), Glomus deserticola (DES), Glomus intraradices (INT), Glomus etunicatum (ETU), or the control (CON) on the shoot dry weight and root fresh weight of Siratro at two harvests. Bars represent the means of five replicates. Means with the same letter within a harvest are not different ($P < 0.05$).

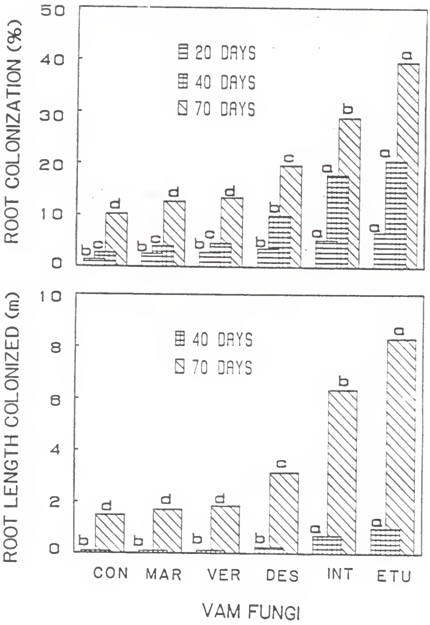

Fig. 4-2 Effect of inoculation with Gigaspora margarita (MAR), Glomus versiforme (VER), Glomus deserticola (DES), Glomus intraradices (INT), Glomus etunicatum (ETU), or the control (CON) on the percentage of root colonization and root length colonized of Siratro at three and two harvests, respectively. Bars represent the means of five replicates. Means with the same letter within a harvest are not different ($P < 0.05$).

colonization ($r^2 = 0.83^{**}$). There was a quadratic relationship between shoot dry weight and length of Siratro root colonized by VAM fungi for all inoculated treatments (Fig. 4-3).

Plant height and number of leaves per plant were not different among treatments at any harvest. After 70 d, the mean plant height and number of leaves for all treatments were 90 and 15 cm, respectively.

There were 2 propagules per gram of soil in the native soil as determined by the MPN test at the beginning of the experiment.

Inoculum density is known to influence plant growth response to VAM fungal inoculation (Hass and Krikum, 1985; Wilson, 1984). Thus, one of the problems in comparing the efficacy of VAM fungi is ensuring uniform inoculum densities (Daniels et al., 1981). In this study, I used the MPN technique to provide a measure of the inoculum densities of the VAM fungi, and I adjusted the inoculum densities so that they were uniform for all inoculated treatments.

There were striking differences in the effectiveness of VAM fungi on Siratro. The results are consistent with the findings of others (Miller et al., 1985; Schubert and Hayman, 1986) indicating that different species and strains of VAM fungi vary considerably in the benefits they confer to the host plant. This experiment also confirmed previous work (Chapter III) which demonstrated that the native population of VAM fungi in this soil was less able to stimulate the growth of Siratro than effective, introduced species. It is possible that the decreasing soil acidity obtained by liming changed the native population of VAM fungi from effective to ineffective as compared to _G. etunicatum_ and _G. intraradices_ (Hayman and Tavares, 1985). Since it is necessary to lime

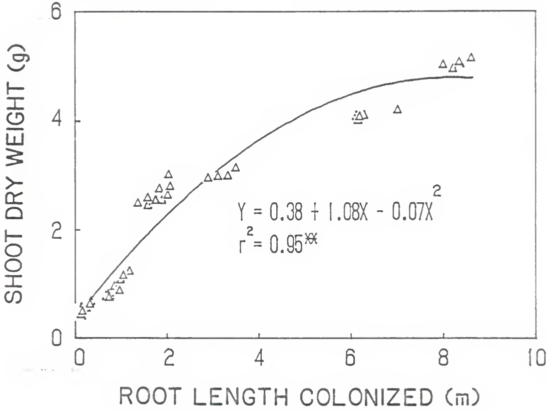

Fig. 4-3 Relationship between shoot dry weight and length of Siratro roots colonized by VAM fungi for all inoculated treatments.

this soil for satisfactory establishment and growth of legumes (Snyder et al., 1985), VAM fungi must be selected for their effectiveness under amended soil conditions.

Powell (1980a) reported a relationship between the level of native inoculum density in the soil and plant growth response to mycorrhizal inoculation. When inoculum density was low (0.01-0.09 propagules g^{-1} soil), there was a significant plant growth response to inoculation with VAM fungi. When inoculum density was higher (0.15-0.30 propagules g^{-1} soil), there was little plant growth response to fungal inoculation. Likewise, good plant growth responses to inoculation with VAM fungi in soils with few indigenous endophytes have been reported by Mosse (1977) and Hall (1979). Thus it would seem that the most promising sites for inoculation with VAM fungi are those where indigenous populations of VAM fungi are very low. However, in this study, where the native inoculum density was relatively high (2 propagules g^{-1} soil), Siratro responded to inoculation with two of the four VAM fungi tested. In addition to the abundance of the indigenous VAM fungi, information about their infectivity and effectiveness is needed to assess potential sites for responsiveness to inoculation with effective VAM fungi.

The ineffectiveness of _G. versiforme_ and _G. margarita_ could be due to an innate symbiotic inefficiency, incompatibility, lack of competitiveness, or to inhibitory edaphic (e.g. soil pH or P level) or environmental factors (e.g. light and temperature). Hayman and Tavares (1985) showed clearly that different endophytes vary in their symbiotic effectiveness at different soil acidities. In addition, some endophytes may be less effective on certain plant hosts. For example,

Schroder et al. (1977) reported that G. macrocarpum increased growth of onions but decreased growth of Stylosanthes sp.

The most effective fungi in this study were those that colonized the root most rapidly. Sanders et al. (1977) and Abbott and Robson (1978) reported that VAM fungi differ in their rates of root colonization. Abbott and Robson (1981) stated that differences in the effectiveness of VAM fungi could be due to differences in their ability to (1) colonize the roots rapidly (infectivity), (2) produce external hyphae, and (3) to take up and transport P (efficiency). In this study, we measured only root colonization over time (infectivity) and found, under the conditions of this experiment, G. etunicatum was the most infective fungus.

Mycorrhizal root colonization, expressed as either percentage or total root length colonized, was positively correlated with the shoot dry weight of Siratro. Abbott and Robson (1981) and Plenchette et al. (1982) also showed positive correlations between the magnitude of mycorrhizal root colonization and shoot dry weights of plants grown on P-deficient soils. These results suggest that differences in endophyte effectiveness may be evaluated on the basis of rates of root colonization. However, Hayman and Tavares (1985) demonstrated that final root colonization by VAM fungi may give little indication of the ability of an endophyte to stimulate plant growth. Abbott and Robson (1978) reported that VAM fungi which differ in effectiveness and rate of root colonization may have similar plateau levels of colonization at a late harvest. Therefore it is not surprising, when colonization is assessed at a relatively advanced stage of plant growth, that there is often little correlation between mycorrhizal root colonization and effectiveness.

Forage legumes are an important component of improved pastures and must be established rapidly and without excessive cost. When P is a major factor limiting the productivity of legumes, large applications of P fertilizer are normally required for legume establishment. However, with the increasing cost of P fertilizer, alternative strategies for minimum fertilizer input and efficient use must be adopted. These data demonstrate that by careful selection of effective VAM fungi, the growth of Siratro can be enhanced in a P-deficient native soil containing a less effective native VAM population than the introduced VAM fungi.

CHAPTER V

GROWTH RESPONSE OF SIRATRO TO INOCULATION WITH VA MYCORRHIZAL FUNGI IN NONPASTEURIZED SOIL. II. EFFICACY OF SELECTED VA MYCORRHIZAL FUNGI AT DIFFERENT P LEVELS.

Introduction

Forage legumes are an important component of improved grass pastures. The legumes serve both to increase forage quality and decrease the need for fertilizer N through N_2 fixation. Snyder et al. (1985) studied the responsiveness of several tropical legumes, including Siratro, to P and lime in a typical Florida Spodosol. They reported that lime and P rates of approximately 3000 and 75 kg ha^{-1} produced maximum yield and maximum economic return.

In an experiment with red clover in a field containing 10 mg $NaHCO_3$-soluble P kg^{-1} soil, plants showed an early response to superphosphate, but by the end of the second year yields were high in all plots, equivalent to around 15 t ha^{-1} dry matter (Hayman et al., 1981). This result was attributed to one of the introduced endophytes, G. caledonium, which had spread and sporulated profusely throughout all the plots (including those inoculated with two other endophytes) and had previously enhanced growth of lucerne at this site. In upland pastures in Wales, Hayman and Mosse (1979) found that inoculation of white clover seedlings with a combination of G. Mosseae and G. fasciculatum "E3" in field plots given the standard dressing of 90 kg P ha^{-1} as basic slag doubled plant

growth and greatly enhanced tissue P content and nodulation. Growth responses at other sites varied from large to slightly negative, probably governed in part by the effectiveness of the indigenous VAM population (Hayman and Hampson, 1979).

Species (Miller et al., 1985; Schubert and Hayman, 1986; Thompson et al., 1986), and isolates within a species (Cooper, 1978), of VAM fungi can colonize plants at different rates. If the mycorrhizal growth response is related to the amount of early root colonization (Abbott and Robson, 1981; Chapter IV), then isolates of VAM fungi that colonize roots rapidly, at P levels found in established agricultural soils, may be most suitable for pasture inoculation.

Schubert and Hayman (1986) indicated that, in order to achieve a rational and effective use of inoculants, precise information on the performance of endophytes in soil amended with P was necessary. It is evident that the effect of soil P on symbiosis varies with the specific host and endophyte. Therefore, more research is needed to develop uniform and predictable endophyte-host responses.

In another study described in Chapter IV, *G. etunicatum* and *G. intraradices* were found to be the most effective growth enhancers (out of 5 isolates) of Siratro in a soil amended with a moderate level of P and lime. In the present study, the objective was to evaluate the infectivity and effectiveness of these two fungi over a practical range of applied P.

Materials and Methods

The chemical properties of the soil used, liming, and fertilizer amendments are described previously (Chapter III).

Siratro was used as the host plant in this experiment. The VAM fungi tested were *G. etunicatum* (isolate S312) and *G. intraradices* (isolate S313). These isolates were maintained in pasteurized soil in pots with bahiagrass as the host. Soils from 10-week-old pot cultures were used to inoculate experimental pots. The propagule densities of the inocula were determined by the MPN technique (Daniels and Skipper, 1982) and approximately 240 propagules were added to each 15-cm-diam plastic pot. Details on the origin of the two fungal isolates were reported previously (Chapter II, Table 2-4). Fungal inoculation technique, planting, and watering were described in Chapter III.

The experiment was designed as a 3 x 4 factorial consisting of 3 inoculation treatments; *G. intraradices*, *G. etunicatum*, and noninoculated control, and four P treatments; 2.5, 10, 20, and 40 mg P kg^{-1} as $Ca(H_2PO_4)_2.H_2O$ (equivalent to 5, 20, 40, and 80 kg P ha^{-1} assuming a 15-cm depth of soil ha^{-1} with a bulk density of 1.3 g $cm^{-3)}$. Phosphorus was applied in solution one week before planting. Soil samples from each treatment were analyzed for extractable P at the beginning and end of the experiment using the Mehlich-I method (0.05 *M* HCl + 0.0125 *M* H_2SO_4). The twelve treatments were replicated five times and arranged on a greenhouse bench in a randomized complete block design.

The average maximum and minimum greenhouse temperatures during the experimental period were 34 and 26°C, respectively. Maximum photosynthetic photon flux density was 1800 μ mol m^{-2} s^{-1}.

Shoot dry and root fresh weights, and percentage and total root lengths colonized by VAM fungi, were determined after 60 d using procedures described previously (Chapter IV). Data for all variables were subjected to ANOVA procedures and regression analysis (SAS Institute Inc., 1982).

Results and Discussion

Phosphorus applications of 2.5, 10, 20, and 40 mg kg^{-1} resulted in Mehlich-I extractable P in the soil of 5.6, 12.8, 21.6, and 38.0 mg kg^{-1} at the beginning of the experiment, respectively. These differences were still reflected at the end of the experiment when P concentrations were 4.4, 7.8, 15.2, and 24.6 mg kg^{-1}.

Shoot dry and root fresh weights of Siratro were increased by P fertilization and fungal inoculation (Fig. 5-1). At 2.5 mg P kg^{-1}, there was no difference in the shoot dry and root fresh weights among inoculated and control plants. At all other levels of P, inoculated plants had higher shoot dry and root fresh weights than control plants. Shoot dry weights were greatest for plants inoculated with _G. etunicatum_. There were no differences in root fresh weights between plants inoculated with _G. etunicatum_ or _G. intraradices_. Inoculated plants had a quadratic relationship for shoot dry and root fresh weights and P application

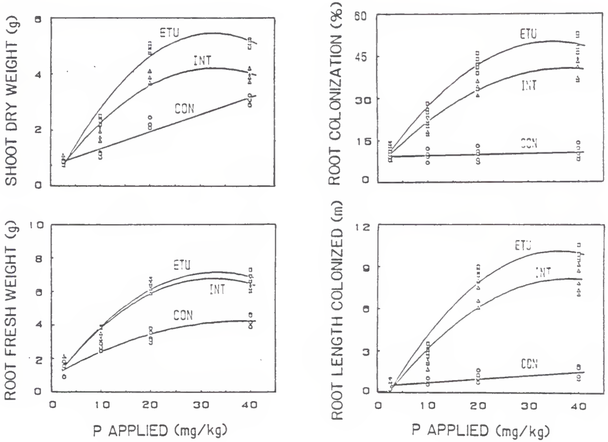

Fig. 5-1. Effect of P application on shoot dry weight, root fresh weight, percentage of root colonized by VAM fungi, and total root length colonized of Siratro grown in limed nonpasteurized soil and inoculated with *Glomus etunicatum* (ETU), *Glomus intraradices* (INT), or not inoculated (CON).

(Table 5-1). Maximum yield of inoculated plants was achieved between 28 and 30 mg kg^{-1} of applied P. Control plants had a linear relationship for shoot dry weights and a quadratic relationship for root fresh weights. There were fungus x P interactions for shoot dry and root fresh weights (Table 5-2).

Percentage and total root length colonized by VAM fungi for the inoculated treatments increased with P additions (Fig. 5-1). This effect was greater for G. etunicatum than for G. intraradices. Phosphate application did not alter the percentage and total root length colonized in the control plants. Inoculated plants had a quadratic relationship for percentage and total root length colonized and applied P (Table 5-1). Maximum colonization of inoculated treatments, expressed as either percentage or total length of colonized root, was attained between 32 and 35 mg kg^{-1} of applied P. There were fungus x P interactions for percentage and total root length colonized by VAM fungi (Table 5-2).

Shoot dry weight of Siratro over the range of applied P was highly correlated with percentage ($r^2 = 0.95^{**}$) and total root length colonized by VAM fungi ($r^2 = 0.97^{**}$) (Fig. 5-2) for both inoculated treatments. Percentage of the root colonized by VAM fungi was very closely correlated ($r^2 = 0.98^{**}$) with the total root length colonized.

Growth enhancement from VAM inoculation at different levels of P has been reported to vary with VAM fungi (Hayman and Hampson, 1979; Hayman and Mosse, 1979; Schubert and Hayman, 1986; Thompson et al., 1986). For example, Schubert and Hayman (1986) indicated that, when large amounts of P were added (more than 100 mg kg^{-1}), G. mosseae, G. versiforme, G.

Table 5-1. Regression equations and coefficients of determination (r^2) showing the relationship of P level to shoot dry weights, root fresh weights, percentage and total root length colonized.

Variable	Regression equations	r^2
	Glomus etunicatum	
Shoot dry weight (g)	$= -0.12+0.34P-0.006P^2$	0.97^{**}
Root fresh weight (g)	$= 0.50+0.40P-0.006P^2$	0.96^{**}
Root colonization (%)	$= 4.47+2.62P-0.04P^2$	0.97^{**}
Root length colonized (m)	$= -1.43+0.64P-0.009P^2$	0.96^{**}
	Glomus intraradices	
Shoot dry weight (g)	$= 0.16+0.24P-0.004P^2$	0.95^{**}
Root fresh weight (g)	$= 0.55+0.38P-0.006P^2$	0.94^{**}
Root colonization (%)	$= 5.42+1.86P-0.03P^2$	0.95^{**}
Root length colonized (m)	$= -1.08+0.49P-0.006P^2$	0.91^{**}
	Control	
Shoot dry weight (g)	$= 0.72+0.06P$	0.93^{**}
Root fresh weight (g)	$= 0.89+0.17P-0.002P^2$	0.92^{**}

P = phosphorus level **significant at $P < 0.01$

Table 5-2. Analysis of variance for shoot dry weights, root fresh weights, and percentage and total root length colonized.

Source of variation	DF	Shoot MS	root MS	root colonized MS	
				percentage	length
Block	4	0.07**	0.04	7.39	0.069
Fungi (F)	2	11.07	18.01	2650.42	114.13
P rates (P)	3	34.19	59.37	1583.51	118.66
linear (Pl)	1	85.40	146.43	4156.00	305.28
quadratic (Pq)	1	13.97	30.10	577.33	32.23
Cubic (Pc)	1	3.20	1.57	17.14	2.48
F x P	6	1.65**	2.55**	378.46**	22.50**
F x Pl	2	2.58**	4.02**	965.54**	55.69**
F x Pq	2	2.29**	2.08**	159.17**	7.82**
F x Pc	2	0.08	1.55	10.67	3.99
Error	44	0.02	0.13	6.27	0.24

**Significant at $P < 0.01$ MS = mean square

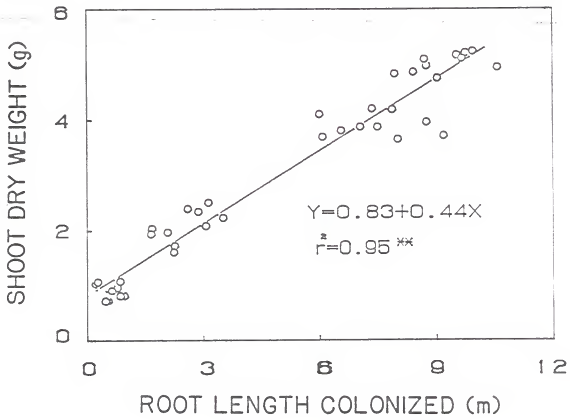

Fig. 5-2. Relationship between shoot dry weight and length of Siratro roots colonized by VAM fungi for all inoculated treatments in nonpasteurized soil.

macrocarpum and G. margarita were ineffective in stimulating growth of onion; however, G. caledonium and Glomus sp. 'E3' were generally effective at all P levels. In our study, G. etunicatum was more effective than G. intraradices at all but the lowest applied P level. Hence, there appears to be good potential for the selection of VAM fungi to enhance plant growth under amended soil conditions such as P fertilization and liming.

Phosphorus has been reported to increase, decrease or not affect root colonization by VAM fungi. However, it is difficult to compare results concerning the effect of P fertilization on mycorrhizal root colonization, because of differences in the range of added P, as well as other factors such as host plant and soil type. In this study, we used a range of 2.5 to 40 mg P kg^{-1} because it represented P levels used in the production of tropical forage legumes in Florida on a similar soil (Snyder et al., 1985). At the lowest P level, G. etunicatum and G. intraradices did not colonize the root or improve growth of Siratro above that of the control plants. Barber and Lougham (1967) reported that, at a very low P level, competition for P occurs between plants and microflora. Habte and Manjunath (1987) and Same et al. (1983) indicated that the growth of VAM fungi is limited by P at very low levels. Between 10 to 40 mg P kg^{-1}, percentage and total root length colonized by VAM fungi increased with P additions. These results agree with those of Abbott and Robson (1977b), Schubert and Hayman (1986), and Thompson et al. (1986), who reported an increase in the percentage and total root length colonized between 18 to 55 mg P kg^{-1}. At high P levels (more than 100 mg kg^{-1}), which are not feasible for field production of

forage legumes, percentage and total root length colonized by VAM fungi may be suppressed (Abbott and Robson, 1977b; Schubert and Hayman, 1986; Thompson et al., 1986).

The result that root colonization by VAM fungi, expressed as either percentage or total root length colonized, is positively correlated to shoot dry weight is consistent with the findings of Abbott and Robson (1981) and a previous study where its implications are discussed (Chapter IV).

I conclude that, in amended soils where the indigenous population of VAM fungi is less effective than some of the introduced species of VAM fungi, inoculation with effective VAM fungi can increase the plant growth. Furthermore, with highly mycorrhizal dependent crops such as tropical legumes, growth enhancement may occur at P levels actually used in commercial pasture production.

CHAPTER VI
EFFECT OF INOCULATION WITH *GLOMUS ETUNICATUM* ON THE GROWTH AND UPTAKE OF P AND N OF *MACROPTILIUM ATROPURPUREUM*, *STYLOSANTHES GUIANENSIS*, AND *AESCHYNOMENE AMERICANA*

Introduction

Mycorrhizal colonization is important for legumes because it increases their P uptake (Abbott and Robson, 1977b; Saif, 1987), and therefore nodulation and N_2-fixation (Asimi et al., 1980; Bergersen, 1971; Gates and Wilson, 1974; Gibson, 1976).

Crush (1974) found that VAM fungi increased the growth and nodulation of *Centrosoma pubescens* Benth, Stylo, and *Trifolium repens* L. Mosse et al. (1976) showed that effective nodulation of *Centrosema*, *Stylosanthes*, and *Trifolium* plants in a P-deficient Brazilian Cerrado soil could be achieved only by introducing both VAM fungi and P. Mycorrhizal fungi also have been shown to increase nodulation, N_2-fixation, plant growth, plant N and P content in *Vigna unguiculata* (Islam et al., 1980; Sanni, 1976), *Medicago sativa* (Barea et al., 1980), *Pueraria phaseoloides* and Stylo (Waidyanatha et al., 1979), *Stylosanthes scabra* (Purcino and Lynd, 1985), leucaena (Munns and Mosse, 1980; Purcino et al., 1986), and Siratro (Lynd et al., 1985). However, effective tripartite symbiosis (legume-rhizobium-VAM fungus) is influenced by soil and climatic conditions (Waidyanatha et al., 1979),

which are apparently species-specific (Burt and Miller, 1975; Mosse, 1972).

In a previous study (Chapter V), G. etunicatum was an effective growth enhancer of Siratro in a soil similar to the one used in the present study, with a moderate level of applied P (20-40 mg P kg^{-1}).

The objective of this investigation was to determine the effect of inoculation with a VAM fungus, G. etunicatum, on the growth and plant uptake of P and N of three forage legumes at different P levels in pasteurized soil under greenhouse conditions.

Materials and Methods

The soil used in this investigation, liming, and basic fertilization are described previously (Chapter III). The soil chemical characteristics before soil fertility treatments and after pasteurization (70°C for 4 h) were: pH 4.4 (soil:H_2O=1:2); 1.4% organic matter; 2, 65, 11, and 12 mg kg^{-1} (Mehlich-I extractable) of P, Ca, Mg and K, respectively.

At planting three phosphorus levels were established by application in solution of 12.5, 25, and 50 mg P kg^{-1} as $Ca(H_2PO_4)_2 \cdot H_2O$ which is equivalent to 25, 50, and 100 kg P ha^{-1}, assuming a 15-cm depth of soil ha^{-1} with a bulk density of 1.3 g cm^{-3}.

Glomus etunicatum (isolate S312) was isolated from carpon desmodium at the Agricultural Research and Education Center, Ona, FL. (Chapter II, Table 2-4). Fungal inoculum was produced in pot culture in pasteurized

soil containing bahiagrass. The fungal inoculation technique was reported in Chapter III.

The legume species used in this experiment were: Siratro, aeschynomene, and Stylo. Seeds were scarified with sandpaper, wetted, and sprinkled with type El "cowpea" inoculum (Nitragin Co., Milwaukee, WI) prior to planting. Three germinated seeds were planted per 620 ml "Deepots" (J.M. McConkey & Co, Inc, Summer, WA). After emergence, seedlings were thinned to one per pot.

The experiment was conducted as a 2 x 3 x 3 factorial consisting of 2 inoculation treatments, G. etunicatum and noninoculated control; three P levels, 12.5, 25, and 50 mg kg^{-1}; the 3 legume species; and 3 replications. The 18 treatments were arranged on a nonshaded greenhouse bench in a completely randomized design. The average maximum and minimum greenhouse temperatures during the experimental period were 28 and $20^{o}C$, respectively, and the average maximum photosynthetic photon flux density was 1793 u mol m^{-2} s^{-1}.

After 65 d plants were harvested, shoots dried (70°C for 24 h), weighed, and ground in a Wiley mill using a 20-mesh screen. Shoots were digested by the sealed-chamber procedure of Anderson (1986) and analyzed for P on a Jarrel-Ash 955 inductively-coupled argon plasma spectrometer (ICAP). For nitrogen analysis, samples were digested using a modification of the aluminum block digestion procedure of Gallaher et al. (1975) and ammonia in the digestate was determined by semiautomated colorimetry (Hambleton, 1977). Roots were washed from the soil, air-dried, and weighed. In addition, percentage and total root

length colonized were estimated as described in Chapter IV. A 0-4 scale was used to estimate the number of nodules per plant, with 1 = 20-50, 2 = 50-100, 3 = 100-150, and 4 = > 150 nodules. Data were subjected to ANOVA procedures and regression analysis (SAS institute Inc., 1982).

Results and Discussion

At harvest, both fungal inoculation and P applications increased shoot dry weight, plant P concentration, and total plant P and N of the three legumes (Table 6-1). Root fresh weight was increased for Stylo and Siratro but not for aeschynomene. There were fungus x P interactions for shoot dry and root fresh weights, and total plant N of Stylo. Siratro had fungus x P interactions for shoot dry weight and total plant P and N, whereas aeschynomene only had fungus x P interaction for shoot dry weight.

At all levels of applied P, shoot dry and root fresh weights and total N of Stylo were greater for mycorrhizal plants than nonmycorrhizal plants (Fig. 6-1). Differences between mycorrhizal and nonmycorrhizal plants were most pronounced at intermediate levels of applied P and diminished with further P addition.

Phosphorus concentration and total P of Stylo were not affected by fungus x P interactions (Table 6-1), but there was an overall effect of fungal inoculation (Fig. 6-1). Saif (1987), Mosse et al. (1976), and Waidyanantha et al. (1979) also reported an increase in plant growth, P concentration, and total P and N of Stylo in a pasteurized low P soil following inoculation with VAM fungi and P applications.

Table 6-1. Mean squares and levels of significance from the analysis of variance for shoot dry weight, root fresh weight, P concentration, and total P and N uptake of forage legumes.

Source of Variation	DF	Shoot dry wt	Root fresh wt	P conc	Total P	Total N
			Stylosanthes guianensis			
Fungus	1	0.45	1.13	0.011**	6.93**	500.97
P rates	2	0.64	0.21	0.016**	9.13**	790.32
Lineal	1	1.20	0.36	0.032**	17.69**	1511.78
Quadratic	1	0.09	0.05	0.0004	0.57	68.86
Fungus x P	2	0.046**	0.15**	0.00007	0.65	47.14*
Error	12	0.0057	0.026	0.012	0.18	8.48
			Macroptilium atropurpureum			
Fungus	1	1.40	0.58**	0.0076*	33.78	2360.30
P rates	2	7.28	1.81**	0.028**	163.11	10469.14
Lineal	1	14.54	3.55**	0.056**	325.94	20925.10
Quadratic	1	0.0072	0.05	0.0001	0.28	13.18
Fungus x P	2	0.54**	0.10	0.0014	10.37*	625.52**
Error	12	0.029	0.027	0.00082	2.47	43.27
			Aeschynomene americana			
Fungus	1	0.13	0.042	0.0068**	6.06**	129.34*
P rates	2	0.56	1.07**	0.034**	29.75**	524.16**
Lineal	1	1.09	2.01**	0.064**	58.70**	1035.46**
Quadratic	1	0.027	0.12*	0.0022	0.80	12.85
Fungus x P	2	0.037*	0.030	0.00009	0.10	43.35
Error	12	0.0076	0.015	0.00052	0.25	21.75

*Significance at $P < 0.05$ **Significant at $P < 0.01$

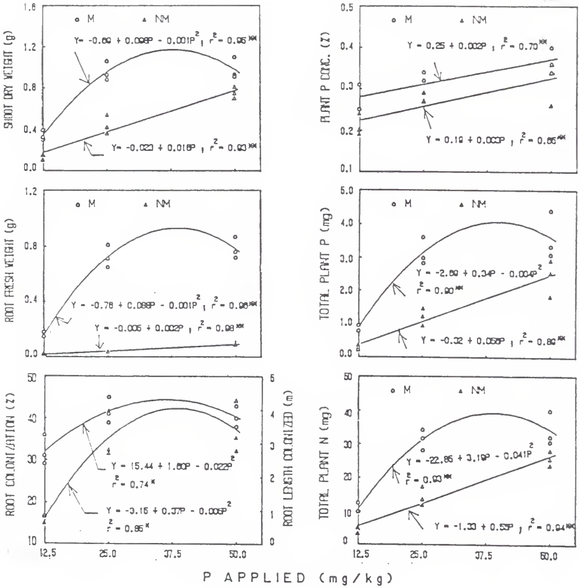

Fig. 6-1. Effect of fungal inoculation and P applications on the shoot dry weight, root fresh weight, root colonization, P concentration, total P, and total N of *Stylosanthes guianensis*.

Mycorrhizal plants of Siratro had greater shoot dry weight, total P and total N than nonmycorrhizal plants at low and intermediate levels of applied P, but not at the highest level (Fig. 6-2). Overall, mycorrhizal plants had greater root fresh weight and plant P concentration than nonmycorrhizal plants. Other investigators (Lynd et al., 1985; Saif, 1987) have shown similar responses of Siratro to inoculation with VAM fungi and P additions. Differences in shoot dry weight between mycorrhizal and nonmycorrhizal plants of aeschynomene were only at the intermediate level of applied P (Fig. 6-3). Fungal inoculation did not affect the root fresh weight. Overall, mycorrhizal plants had greater P concentration, total P, and total N than nonmycorrhizal plants (Fig. 6-3).

Percentage and total root length colonized for mycorrhizal plants of Stylo (Fig. 6-1), Siratro (Fig. 6-2), and aeschynomene (Fig. 6-3) increased with the first addition of P. However, maximum colonization, expressed as either percentage or total length of colonized root of the three legumes, was attained at the intermediate levels of applied P.

The number of nodules in the three legumes increased with fungal inoculation and P applications. Mycorrhizal plants of Stylo had more nodules than nonmycorrhizal plants at all levels of applied P. However, mycorrhizal plants of Siratro and aeschynomene had more nodules than nonmycorrhizal plants only at 25 mg kg^{-1} of applied P.

Mycorrhizal plants required between 38-40 mg P kg^{-1} to achieve maximum shoot dry weight, whereas nonmycorrhizal plants required 50 mg P kg^{-1} to produce approximately the same shoot dry weight, except for nonmycorrhizal Stylo (Fig 6-1) which even with 50 mg P kg^{-1} did not

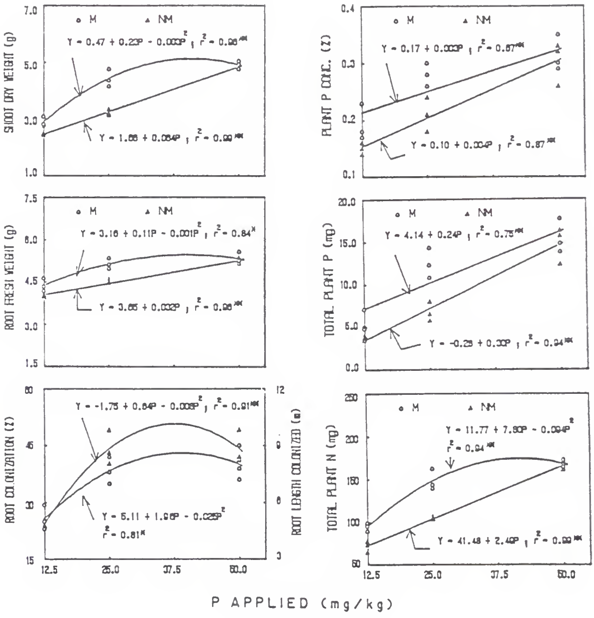

Fig. 6-2. Effect of fungal inoculation and P applications on the shoot dry weight, root fresh weight, root colonization, P concentration, total P, and total N of <u>Macroptilium atropurpureum</u>.

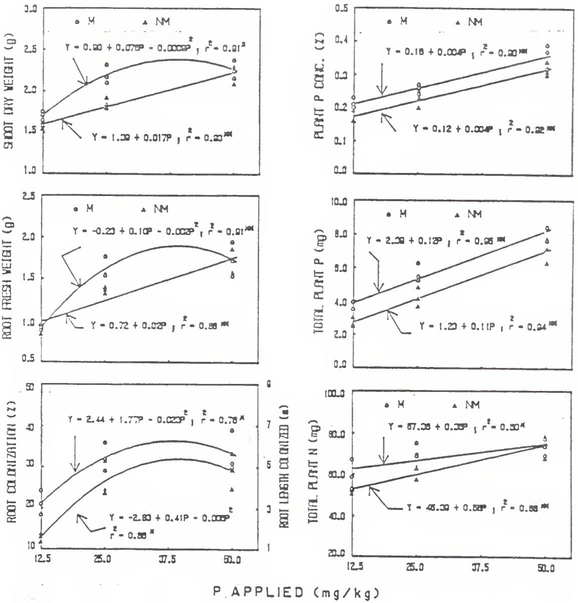

Fig. 6-3. Effect of fungal inoculation and P applications on the shoot dry weight, root fresh weight, root colonization, P concentration, total P, and total N of *Aeschynomene americana*.

reach the same shoot dry weight as mycorrhizal plants. Fungal inoculation with G. etunicatum resulted in a 20% decrease in the amount of P required for maximum yield. This demonstrates the importance of VAM fungi in the P nutrition of tropical legumes and would represent an important savings to the farmer.

Growth responses of Stylo, Siratro, and aeschynomene associated with inoculation with VAM fungi are closely related to improved P uptake and N_2-fixation. These results clearly support the findings of earlier studies that inoculation with VAM fungi not only stimulates plant growth and P uptake of legumes (Abbott and Robson, 1977b; Habte and Manjunath, 1987; Menge, 1983) but also nodulation and N_2-fixation (Asimi et al., 1980; Lynd et al., 1985; Purcino et al., 1986) which was measured indirectly in this study by the total plant uptake of N.

Inoculation with effective species of VAM fungi and additions of P between 25 and 50 mg kg^{-1}, were shown to improve the growth and uptake of P and N of Stylo, Siratro, and aeschynomene.

CHAPTER VII
GROWTH RESPONSE OF MACROPTILIUM ATROPURPUREUM AND AESCHYNOMENE AMERICANA TO INOCULATION WITH SELECTED VA MYCORRHIZAL FUNGI IN THE FIELD AT DIFFERENT P LEVELS

Introduction

The practical goal of studies on plant growth responses to inoculation with VAM fungi is to obtain increased yield of plants growing under field conditions. Significant plant growth responses to inoculation with VAM fungi have been demonstrated in pot experiments using pasteurized and nonpasteurized soil for several tropical forage legumes such as: *Pueraria phaseoloides* (Salinas et al., 1985; Waidyanatha et al., 1979), leucaena (Habte and Manjunath, 1987; Huang et al., 1985), Siratro (Lynd et al., 1985), Stylo (Mosse, 1977) and aeschynomene (Chapter II and VI).

It has been pointed out that inoculation experiments with VAM fungi should include testing a series of P levels (Abbott and Robson, 1977b; Hall, 1978; Powell, 1980b) in order to select the optimum P level for a mycorrhizal response. Except for the work by Saif (1987), little information is available on plant growth response of tropical forage legumes to inoculation with VAM fungi in nonpasteurized soil under field conditions at different P levels. However, there is more data for temperate legumes. In general, the field sites where plants are most likely to respond to inoculation with VAM fungi are those containing

little soluble phosphate and a small or ineffective native population of VAM fungi. The experimental site selected for this study contains very little available P (1 mg kg^{-1}) and a reasonably high indigenous population of VAM fungi (2 propagules g^{-1} soil). The indigenous population, however, was less effective than two of the five introduced VAM fungi under amended soil conditions (Chapter IV).

Aeschynomene is used extensively in Florida as a forage legume to supply fixed nitrogen as protein and minerals to grazing animals (Hodges et al., 1982) Siratro has been used sparingly in Florida (Kretschmer, 1972), but is one of the most widely used legumes throughout tropical regions of the world (Lynd et al.,1985). However, for the satisfactory establishment and growth of aeschynomene and Siratro in highly acidic and P-deficient soils, lime and P fertilizer must be applied (Snyder et al., 1985). Recent greenhouse experiments have shown that better growth of forage legumes in these soils can be achieved by inoculation with selected species of VAM fungi (Chapter IV and V). Glomus etunicatum and G. intraradices were found to be effective growth enhancers of Siratro and aeschynomene in a nonpasteurized, limed (3000 kg ha^{-1}) soil similar to the one used in the present study, over a range of 20-80 kg ha^{-1} of applied P. It is therefore of considerable interest to determine whether inoculation with selected isolates of VAM fungi can improve the establishment, growth, and nutrient uptake of Siratro and aeschynomene, under field conditions where soil was limed and fertilized with different P levels.

Materials and Methods

The soil used was a native Oldsmar fine sand (sandy, siliceous, hyperthermic Alfic Arenic Haplaquods) with a pH of 4.6 (soil:H_2O=1:2), 1.5% organic matter, and the following Mehlich-I extractable elements in mg kg^{-1}: P - 1.0, Ca - 65, Mg - 12, and K - 15.

The experiment was designed as a 2 x 3 x 4 factorial consisting of two legume species; Siratro and A. americana; three inoculation treatments, G. etunicatum, G. intraradices, and the control; and four P treatments; 10, 30, 60, and 120 kg ha^{-1} as triple superphosphate. The 24 treatments were arranged in a randomized complete block design with ten replications per treatment. The P treatments were surface applied by hand on 3 July 1986, along with a basal application of lime (high calcitic limestone), Mg, nutritional spray (Diamond Fertilizer Co., Ft. Pierce, FL.), and Mo at 3000, 25, 22, and 0.2 kg ha^{-1}, respectively, and incorporated using a rake to a depth of approximately 15 cm. Potassium was broadcasted on each plot at a rate of 60 kg ha^{-1} as KCL on 5 August 1986.

Seeds of Siratro and aeschynomene inoculated with rhizobium type El "Cowpea" inoculum (Nitragin Co., Milwaukee, WI) were sown in pasteurized Oldsmar fine sand, amended with high calcitic limestone at 1500 mg kg^{-1} and P at 12.5 mg kg^{-1}, in cells of "speedling" styrofoam trays (72 cells per tray) on 20 June 1986.

Seedlings were inoculated or not inoculated with G. etunicatum (isolate S312), G. intraradices (isolate S311). The amount of soil-root inoculum used for each VAM fungi was adjusted to give equal inoculum

densities as determined by the MPN technique (Daniels and Skipper, 1982). Approximately 180 propagules were added mid-way down each cell before seeding. Control seedlings received 15 g of a soil-root mixture from nonmycorrhizal pot cultures and the equivalent of 20 kg P ha^{-1}, which was applied in solution 10 d after germination in an attempt to make the P status of the mycorrhizal and nonmycorrhizal seedlings similar at the time of transplanting.

Seeded trays were placed in a glasshouse for 6 wk, after which the whole cell content (each with one seedling) was transplanted to the field. Siratro seedlings were cut back to three nodes each before transplanting. Seedlings were transplanted on 4 August 1986.

One seedling was planted by hand in the middle of each 1.0 by 1.0 m plot which were surrounded by alleyways of 1.0 m. Extra seedlings were weighed, and P concentration and root colonization were determined. Harvests of Siratro and aeschynomene were made on 2 October 1986. Siratro, a perennial, also was harvested on 27 November 1986, 5 May 1987, and 29 June 1987. Herbage was dried at 75°C for 24 h and weighed. Five subsamples per treatment from the first harvest of Siratro and aeschynomene foliage were analyzed for N and P content by automated colorimetry (Technicon Industrial Systems Method No. 334-74 W/B, Technicon Instruments Corp., Tarrytown, NY). Five root samples per treatment, consisting each of four subsamples, were used to assess mycorrhizal root colonization. Percentage of root colonized by VAM fungi of aeschynomene and Siratro (1st and 4th harvests), was estimated as described in Chapter III.

Soil samples (0-15 cm) were taken at the 4th harvest and analyzed for pH, P, Ca, Mg, and K using the Mehlich-I extractant method. All elements were determined by inductively coupled argon plasma (ICAP) spectrometry. Data for all variables were subjected to ANOVA procedures and regression analysis (SAS Institute Inc., 1982).

Results and Discussion

Pre-inoculated seedlings were used in this study to ensure the legume roots were well colonized with the selected VAM fungi, because this was thought to be the most certain way to ensure establishment of the inoculum. Once a plant response is ascertained, methods of inoculation more applicable on a large scale can be tested.

As a result of lime application, soil pH increased from 4.6 to about 6.2, and extractable Ca increased up to about 650 mg kg^{-1}. Phosphorus applications of 10, 30, 60, and 120 kg ha^{-1} resulted in extractable P in the soil of 5.1, 8.6, 17.8, and 34.3 mg kg^{-1} at the end of the experiment, respectively. Thus the legume species and VAM fungi in the present study were exposed to a considerable range of soil P.

Seedlings inoculated with VAM fungi were similar in shoot dry weight and P concentration to control seedlings at transplanting (Table 7-1). Seedlings inoculated with VAM fungi were also well colonized, whereas no VAM colonization was detected on control seedlings (Table 7-1).

Phosphorus amendments and fungal inoculation increased shoot dry weights of aeschynomene (Table 7-2) and all harvests of Siratro

Table 7-1. Shoot dry weights, P concentrations, and percentage of roots colonized of Siratro and aeschynomene seedlings at transplanting.

VAM Inoculation	Siratro^{z}			aeschynomene^{z}		
	Shoot dry wt.	Shoot P	Root colon.	shoot dry wt.	Shoot P	Root colon.
	mg	--- % ---		mg	--- % ---	
G. etunicatum	302	.18	52	304	.17	57
G. intraradices	305	.17	60	299	.20	55
Control	298	.16	0	302	.18	0

^{z}Data are means of five replicates.

Table 7-2. Analysis of variance for shoot dry weights of Aeschynomene americana harvested 2 October 1986.

Source of variation	DF	Mean squares
Block	9	52.57
Fungi (F)	2	1654.72**
Phosphorus (P)	3	6986.26**
Linear (Pl)	1	18700.24**
Quadratic (Pq)	1	2250.32**
Cubic (Pc)	1	8.21
F x P	6	91.79
F x Pl	2	53.72
F x Pq	2	215.53
F x Pc	2	6.11
Error	99	45.83

**Significant at $P < 0.01$

(Table 7-3). There were no fungi x P interactions for shoot dry weights of Siratro or aeschynomene.

At all levels of applied P and at all harvests, shoot dry weights of Siratro were greater for fungal inoculated plants than control plants (Fig. 7-1). Except for the third harvest, where the effect of fungal inoculation was less pronounced at all levels of applied P, differences between fungal inoculated and noninoculated plants were most marked at intermediate levels of applied P (30-90 kg ha^{-1}) and diminished at the highest level (120 kg ha^{-1}). The effect of inoculation with VAM fungi on the shoot dry weights of aeschynomene, at all levels of applied P, followed the same trend as that of Siratro although the response to mycorrhizal inoculation was greater (Fig. 7-2).

Inoculated plants of Siratro (Table 7-4 and 5) and aeschynomene (Table 7-6) had a quadratic relationship between shoot dry weight and P application. Maximum shoot dry weight of Siratro was achieved between 75-85 kg ha^{-1} of P and for aeschynomene at 85 kg ha^{-1} of P, whereas control plants of both legumes, even with 120 kg ha^{-1} of P, did not reach the same shoot dry weight as fungal inoculated plants. Thus inoculation with VAM fungi resulted in at least a 30% savings (40 kg ha^{-1}) in the amount of P fertilizer required for maximum yield. In a previous greenhouse experiment (Chapter VI), I found that inoculation with G. etunicatum resulted in a 20% decrease in the amount of P required for maximum yield of Siratro. Some previous reports of VAM field experiments with legumes in nonpasteurized soil (Black and Tinker, 1977; Khan, 1975) show responses to VAM inoculation only in the absence of P fertilizer.

Table 7-3. Analysis of variance for shoot dry weights from four Siratro harvests.

Source of variation	DF	Mean squares			
		Harvest 1 2 Oct. 86	Harvest 2 27 Nov. 86	Harvest 3 5 May 87	Harvest 4 29 June 87
Block	9(6)[z]	6.40	4.14	11.47	18.70*
Fungi (F)	2	332.20	247.79**	69.20**	177.21**
Phosphorus (P)	3	807.52	1577.91**	1598.20**	1806.27**
Linear (Pl)	1	2107.47	4021.98**	4223.03**	4630.88**
Quadratic (Pq)	1	305.04	711.49**	559.16**	773.57**
Cubic (Pc)	1	10.07	0.23	12.38	14.32
F x P	6	6.35**	3.21	1.67	10.12
F x Pl	2	4.61**	3.89	1.10	7.69
F x Pq	2	12.82**	5.38	2.76	15.83
F x Pc	2	1.63	0.38	1.14	6.83
Error	99(66)	8.89	13.43	9.20	7.98

**Significant at $P < 0.01$ *Significant at $P < 0.05$

[z]Values in parentheses are the degrees of freedom for harvests 2, 3, and 4 for which only 7 replicates were used.

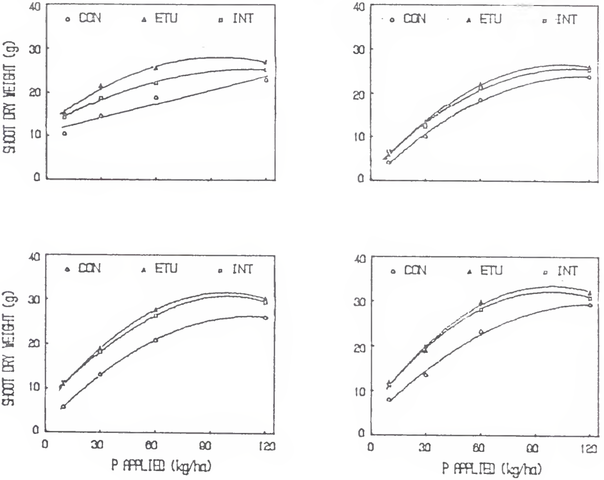

Fig. 7-1. Effect of P application on shoot dry weights for the first (A), second (B), third (C), and fourth (D) harvest of Siratro grown under field conditions and inoculated with Glomus etunicatum (ETU), Glomus intraradices (INT), or not inoculated (CON). Data points are means of five replicates.

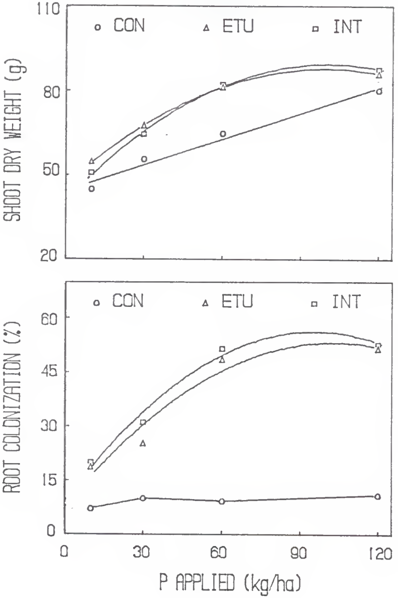

Fig. 7-2. Effect of P application on shoot dry weight and percentage of root colonized of <u>Aeschynomene americana</u> grown in the field and inoculated with <u>Glomus etunicatum</u> (ETU), <u>Glomus intraradices</u> (INT), or not inoculated (CON). Data points are means of five replicates.

Table 7-4. Regression equations and coefficients of determination (r^2) showing the relationship of applied P level to shoot dry weight, percentage of root colonization, P and N concentrations, and total P and N uptake for the first harvest of Siratro.

Variable	Regression equations	r^2
	Glomus etunicatum	
Shoot dry wt. (g)	$= 12.36+0.33P-0.0022P^2$	0.80^{**}
Root coloniz. (%)	$= 5.31+0.77P-0.0043P^2$	0.91^{**}
Plant P conc. (%)	$= 0.22+0.00072P$	0.66^{**}
Total Plant P (mg)	$= 17.73+1.36P-0.0069P^2$	0.98^{**}
Plant N conc. (%)	$= 2.26+0.035P-0.00025P^2$	0.52^{**}
Total Plant N (mg)	$= 170.94+20.09P-0.13P^2$	0.91^{**}
	Glomus intraradices	
Shoot dry wt. (g)	$= 12.03+0.24P-0.0014P^2$	0.67^{**}
Root coloniz. (%)	$= 10.04+0.59P-0.0034P^2$	0.86^{**}
Plant P conc. (%)	$= 0.16+0.0037P-0.000018P^2$	0.76^{**}
Total plant P (mg)	$= 16.08+1.31P-0.0068P^2$	0.94^{**}
Plant N conc. (%)	$= 2.12+0.041P-0.00026P^2$	0.56^{**}
Total plant N (mg)	$= 226.21+15.50P-0.095P^2$	0.91^{**}
	Control	
Shoot dry wt. (g)	$= 10.65+0.11P$	0.65^{**}
Plant P conc. (%)	$= 0.18+0.0011P$	0.81^{**}
Total plant P (mg)	$= 12.36+0.48P$	0.92^{**}
Total plant N (mg)	$= 173.09+3.57P$	0.89^{**}

P = phosphorus level

** Significant at $P < 0.01$

Table 7-5. Regression equations and coefficients of determination (r^2) showing the relationship of applied P level to shoot dry weights for the second, third, and fourth harvest and percentage of root colonization for the fourth harvest of Siratro.

Variable	Regression equation	r^2
	Glomus etunicatum	
Shoot dry wt (g), harvest 2	$= 5.74+0.52P-0.0029P^2$	0.87^{**}
Shoot dry wt (g), harvest 3	$= 0.99+0.48P-0.0029P^2$	0.89^{**}
Shoot dry wt (g), harvest 4	$= 5.98+0.55P-0.0032P^2$	0.87^{**}
Root colon. (%), harvest 4	$= 9.85+0.84P-0.0046P^2$	0.86^{**}
	Glomus intraradices	
Shoot dry wt (g), harvest 2	$= 6.58+0.46P-0.0025P^2$	0.84^{**}
Shoot dry wt (g), harvest 3	$= 2.05+0.43P-0.0025P^2$	0.86^{**}
Shoot dry wt (g), harvest 4	$= 6.14+0.52P-0.0027P^2$	0.87^{**}
Root colon. (%), harvest 4	$= 15.03+0.69P-0.0039P^2$	0.89^{**}
	Control	
Shoot dry wt (g), harvest 2	$= 1.56+0.44P-0.0023P^2$	0.81^{**}
Shoot dry wt (g), harvest 3	$= -0.23+0.42P-0.0021P^2$	0.88^{**}
Shoot dry wt (g), harvest 4	$= 3.38+0.42P-0.0017P^2$	0.91^{**}

P = phosphorus level

**Significant at $P < 0.01$

Table 7-6. Regression equations and coefficients of determination (r^2) showing the relationship of applied P level to shoot dry weight, percentage of root colonization, P concentration, and total P and N uptake of *Aeschynomene americana*.

Variable	Regression equations	r^2
	Glomus etunicatum	
Shoot dry wt. (g)	$= 46.59+0.83P-0.0049P^2$	0.78^{**}
Root coloniz. (%)	$= 7.89+0.88P-0.0047P^2$	0.89^{**}
Plant P Conc. (%)	$= 0.21+0.0037P-0.000019P^2$	0.80^{**}
Total plant P (mg)	$= 67.54+4.96P-0.025P^2$	0.96^{**}
Total plant N (g)	$= 1.35+0.039P-0.00019P^2$	0.93^{**}
	Glomus intraradices	
Shoot dry wt. (g)	$= 40.86+0.97P-0.0056P^2$	0.86^{**}
Root coloniz. (%)	$= 9.26+0.98P-0.0058P^2$	0.93^{**}
Plant P Conc. (%)	$= 0.16+0.0053P-0.000029P^2$	0.76^{**}
Total plant P (mg)	$= 43.24+5.85P-0.031P^2$	0.95^{**}
Total plant N (g)	$= 1.18+0.043P-0.00022P^2$	0.91^{**}
	Control	
Shoot dry wt. (g)	$= 43.50+0.31P$	0.76^{**}
Plant P Conc. (%)	$= 0.11+0.0045P-0.000021P^2$	0.95^{**}
Total plant P (mg)	$= 28.08+3.63P-0.012P^2$	0.99^{**}
Total plant N (g)	$= 1.44+0.015P$	0.81^{**}

P = Phosphorus level ** Significant at $P < 0.01$

Hayman and Mosse (1979), however, reported improved growth of white clover in the field after inoculation with VAM fungi and the addition of 90 kg ha^{-1} of P. They also indicated that responses to fungal inoculation were smaller with 23 kg ha^{-1} of P and were absent where no P was added. Similarly, Hall (1984) reported that inoculation with selected VAM fungi increased yield of white clover in the field only if 50 kg ha^{-1} of P was also applied.

Several greenhouse experiments on the phosphate response curves of fungal inoculated and noninoculated forage legumes have been carried out, mostly using clovers (Abbott and Robson, 1977b; Sparling and Tinker, 1978; Powell, 1980b) and Siratro (Lynd et al., 1985; Medina et al., 1987d) as the test plants. These authors applied soluble P fertilizers at rates ranging from 0 to 250 kg ha^{-1} and have reached the general conclusion that inoculation with VAM fungi markedly increases legume growth at low and intermediate rates of applied P. From the practical point of view, however, the interactions between phosphate additions and VAM on legumes are not always predictable and generalizable, because the responses are modulated by the incidence of several factors. These include the physical and chemical characteristics of the soil, plant species, VAM fungi, and the complex interactions between these factors.

At the first harvest of Siratro (Fig. 7-1A), plants inoculated with G. etunicatum had higher shoot dry weights than plants inoculated with G. intraradices at all levels of applied P. However, in subsequent harvests of Siratro (Fig. 7-1BC and D) and for aeschynomene (Fig. 7-2) the response of shoot dry weight to inoculation with the two VAM fungi was not different.

Phosphorus additions and fungal inoculation increased percentage of root colonized by VAM fungi of Siratro (Table 7-7) and aeschynomene (Table 7-8). There were fungi x P interactions for percentage of root colonized by VAM fungi for for both legumes. Inoculated treatments had greater percentage of root colonized than control treatments at all levels of applied P. Percentage of root colonized by VAM fungi for the inoculated plants of Siratro (Fig. 7-3A and B) and aeschynomene (Fig. 7-2) increased linearly with P additions up to 60 kg ha^{-1}. Phosphorus application of 120 kg ha^{-1} did not affect the percentage of root colonized by VAM fungi. In a previous greenhouse study (Chapter V), I found that percentage of Siratro root length colonized by VAM fungi increased with P additions up to 40 mg kg^{-1}, which is equivalent to 80 kg ha^{-1} of P. Abbott and Robson (1977b) and Schubert and Hayman (1986), also in pot experiments, reported an increase in the percentage of root length colonized up to 55 mg kg^{-1} of P (110 kg ha^{-1}).

Phosphorus applications did not alter the percentage of root colonized in the control plants. The degree of root colonization of the control plants of Siratro increased from 9% in the first harvest to about 19% by the fourth harvest (Fig. 7-3A and B), but still failed to increase the shoot dry weight of the control plants compared to the inoculated plants. Fungal inoculated plants of Siratro (Table 7-4 and 5) had a quadratic relationship between percentage of root colonized and applied P. Maximum root colonization was attained between 85-90 kg ha^{-1} of P for both legumes. At lower P additions, Siratro plants inoculated with G. intraradices had greater percentage of root colonized than plants inoculated with G. etunicatum (Fig. 7-3). However, there were no

Table 7-7. Analysis of variance for percentage root colonized of Siratro by VAM fungi.

Source of variation	DF	Mean squares Harvest 1 2 Oct. 86	Mean squares Harvest 4 29 June 87
Block	4	9.27	14.61
Fungi (F)	2	2394.82	1206.47
Phosphorus (P)	3	935.53	1230.09
Linear (Pl)	1	2195.71	2538.78
Quadratic (Pq)	1	552.25	1083.74
Cubic (Pc)	1	58.62	67.74
F x P	6	230.66**	86.82**
F x Pl	2	510.93**	174.04**
F x Pq	2	112.49**	77.26**
F x Pc	2	68.55**	9.16
Error	44	7.98	11.91

**Significant at $P < 0.01$ *Significant at $P < 0.05$

Table 7-8. Analysis of variance for percentage root colonized, P concentration, and total P and N uptake of *Aeschynomene americana* harvested 2 October 1986.

Source of variation	DF	Mean Squares Root colon.	P conc.	total P	total N
Block	4	10.11	0.00065	384.02	0.051
Fungi (F)	2	5276.62	0.0098**	9052.18	0.39**
Phosphorus (P)	3	1844.64	0.051**	75570.74	4.64**
Linear (Pl)	1	4408.21	0.12**	196270.22	12.04**
Quadratic (Pq)	1	1001.65	0.031**	29179.92	1.89**
Cubic (Pc)	1	124.08	0.0012	1262.07	0.0021
F x P	6	378.66**	0.0010	1190.91**	0.026
F x Pl	2	833.18**	0.0021	1275.75**	0.013
F x Pq	2	220.16**	0.00052	1815.29**	0.043
F x Pc	2	82.64**	0.00021	481.71	0.022
Error	44	11.60	0.0011	206.45	0.062

**Significant at P < 0.01 *Significant at P < 0.05

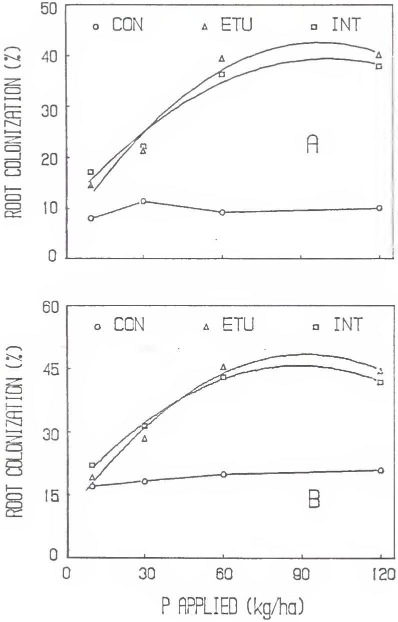

Fig. 7-3. Effect of P application on percentage of root colonized for the first (A) and fourth (B) harvest of Siratro grown in the field and inoculated with *Glomus etunicatum* (ETU), *Glomus intraradices* (INT), or not inoculated (CON). Data points are means of five replicates.

differences in root colonization between G. etunicatum and G. intraradices at any level of applied P. For aeschynomene, differences between G. intraradices and G. etunicatum were only at 30 kg ha^{-1} of applied P (Fig. 7-2).

At the first harvest, P amendments and fungal inoculation also had an effect on P concentration, total P uptake, N concentration, and total N uptake of Siratro (Table 7-9). These same parameters were increased for aeschynomene, except for N concentration (Table 7-8). There were fungi x P interactions for P concentration, total P uptake, N concentration, and total N uptake of Siratro. By the contrast, aeschynomene only had fungi x P interaction for total P uptake.

Inoculated plants of Siratro had greater P concentration, total P uptake, N concentration, and total N uptake than control plants at low and intermediate levels of applied P (Fig. 7-4). At the highest level of applied P, total P uptake of Siratro plants inoculated with G. intraradices and P and N concentrations of plants inoculated with G. etunicatum did not differ from those of control plants. Fungal inoculated plants of aeschynomene also had greater P concentration, total P uptake, and total N uptake than control plants at low and intermediate levels of applied P, but not at the highest level (Fig. 7-5). The positive effect of inoculation with VAM fungi on P and N uptake have been shown for other forage legumes, for example, leucaena (Habte and Manjunath, 1987), Pueraria phaseoloides (Sanchez and Salinas, 1981), and field experiments with white clover (Hall, 1984; Hayman and Mosse, 1979) and Medicago sativa (Azcon-Aguilar and Barea, 1981). Potassium,

Table 7-9. Analysis of variance for P and N concentrations, and total P and N uptake of Siratro for the first harvest.

Source of variation	DF	Mean Squares P Conc.	total P	N Conc.	total N
Block	4	0.00033	26.06	0.42*	10491.95**
Fungi (F)	2	0.0059	2556.03	3.03	540732.85
Phosphorus (P)	3	0.039	7248.85	1.47	544117.23
Linear (Pl)	1	0.11	19691.07	1.53	1006530.96
Quadratic (Pq)	1	0.0049	2055.48	2.87	625338.95
Cubic (Pc)	1	0.000077	0.010	0.010	487.84
F x P	6	0.0023*	175.50**	0.33*	33638.14**
F x Pl	2	0.0031*	53.34	0.21	7291.34
F x Pq	2	0.0039*	465.13**	0.75*	92589.06**
F x Pc	2	0.00011	8.02	0.040	1034.01
Error	44	0.00087	26.84	0.14	3606.65

**Significant at P < 0.01 *Significant at P < 0.05

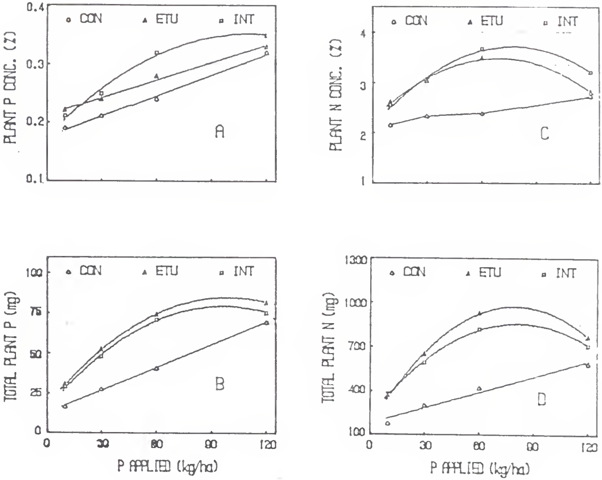

Fig. 7-4. Effect of P application on P concentration, total P, N concentration, and total N of Siratro grown in the field and inoculated with *Glomus etunicatum* (ETU), *Glomus intraradices* (INT), or not inoculated (CON). Data points are means of five replicates.

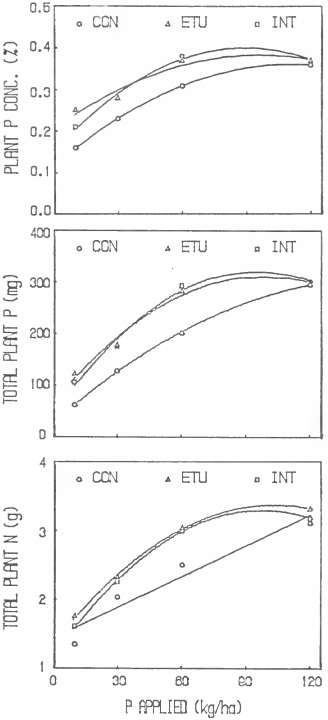

Fig. 7-5. Effect of P application on P concentration, total P, and total N of *Aeschynomene americana* grown in the field conditions and inoculated with *Glomus etunicatum* (ETU), *Glomus intraradices* (INT), or not inoculated (CON). Data points are means of five replicates.

Ca, Mg, and Zn concentrations of Siratro and aeschynomene were not related to P applications or fungal inoculation (data not presented).

In contrast to greenhouse pot experiments, field experiments on inoculation with VAM fungi often have been unsuccessful. It is possibly that the main cause for the satisfactory response to fungal inoculation obtained in the present study was due to a largely ineffective indigenous VAM population as compared to *G. etunicatum* and *G. intraradices* under the amended soil conditions. In previous greenhouse experiments (Chapter IV and V) using a similar nonpasteurized soil than the one used in the present study, I found that *G. etunicatum* and *G. intraradices* were effective growth enhancers of Siratro, even with a reasonably high soil native VAM population. Similarly, Powell et al. (1980b) reported that indigenous VAM fungi were ineffective in many soils and that inoculation by more effective VAM fungi would result in positive responses, even in nonpasteurized soils containing a high indigenous VAM population.

In conclusion, the present study shows that effective inoculation with selected VAM fungi can have an important effect on growth of forage legumes in the field in soils that contain ineffective native VAM populations under amended soil conditions, even at moderate levels of applied P.

CHAPTER VIII
CONCLUSIONS

The objective of this chapter is to summarize the work of the preceding six chapters.

The overall goal of this research project was to improve the establishment phases and growth of tropical forage legumes in newly cleared land at reduced P fertilization through inoculation with effective VAM fungi. In order to accomplish this goal, greenhouse studies were carried out in limed, pasteurized and nonpasteurized Oldsmar fine sand, collected from a newly cleared area at the Agricultural Research and Education Center, Fort Pierce, FL. A field experiment was conducted in the same nonpasteurized soil.

In Chapter II, quantitative data on the amount of root colonization and the species distribution of VAM fungi associated with four cultivated tropical forage legumes from four different locations in south Florida are reported. Differences in percentage of root colonization and total spore density were significant among locations, legume species, and location x legume species interactions. Legume species differed in percentage root colonization and total spore density among locations except for carpon desmodium, which showed no differences among locations in percentage root colonization. The six species of VAM fungi collected in this survey were: *G. heterogama*, *G. margarita*, *G. etunicatum*, *G. intraradices*, *Glomus* sp., and *A. spinosa*.

In Chapter III, the effect of inoculation with G. intraradices on the growth of several tropical forage legumes in P-deficient, nonpasteurized and pasteurized soil under greenhouse conditions is reported. Shoot and root dry weights were increased after inoculation in nonpasteurized soil for Siratro, aeschynomene, Aeschynnomene villosa, Stylosanthes hamata, and Stylo, but not for Arachis sp. and Vigna adenantha, which only responded to inoculation in pasteurized soil.

In Chapter IV, the effect of five species of VAM fungi, G. etunicatum, G. deserticola, G. versiforme, G. intraradices, and G. margarita, on the growth of Siratro in a limed, nonpasteurized soil with an applied P level of 20 mg kg^{-1} under greenhouse conditions was determined. Shoot dry and root fresh weights of plants inoculated with G. etunicatum and G. intraradices were higher than the other VAM fungal treatments and noninoculated plants. In addition, plants inoculated with G. etunicatum had higher shoot dry weights than plants inoculated with G. intraradices. The indigenous population of VAM fungi was reasonably high (MPN = 2 propagules g^{-1} soil); however, plant yields were less than the best VAM treated plants. A positive correlation was found between mycorrhizal root colonization, expressed as either percentage or total root length colonized, and shoot dry weight. Glomus etunicatum colonized roots more rapidly than the other VAM fungi tested.

In Chapter V, the effect of G. etunicatum and G. intraradices, on the growth of Siratro in a limed, nonpasteurized soil, with applied P levels of 2.5, 10, 20, and 40 mg kg^{-1} under greenhouse conditions is reported. At 2.5 mg kg^{-1} of applied P, there was no yield response to inoculation. Above 2.5 mg kg^{-1} of applied P, plants inoculated with

either _G_. _etunicatum_ or _G_. _intraradices_ weighed more than control plants. Inoculated plants required between 28 and 30 mg P kg^{-1} to achieve maximum shoot dry weight, whereas control plants, even with 40 mg P kg^{-1}, did not achieve maximum growth. Shoot dry weight response was better with _G_. _etunicatum_ than with _G_. _intraradices_. For both fungi, increasing P above 2.5 mg kg^{-1} increased the percentage and total root length colonized by VAM fungi.

In Chapter VI, the effect of inoculation with _G_. _etunicatum_, on the growth and uptake of P and N of three forage legumes at applied P levels between 12.5 and 50 mg kg^{-1} in a limed, pasteurized soil under greenhouse conditions is reported. At all levels of applied P, shoot dry and root fresh weights, and total N of Stylo were greater for mycorrhizal plants than nonmycorrhizal plants. The differences were most pronounced at intermediate levels of applied P and diminished at the higher P levels. Mycorrhizal plants of Siratro had greater shoot dry weight, total P and N than nonmycorrhizal plants at low and intermediate P levels, but not at the highest level. Differences in shoot dry weight between mycorrhizal and nonmycorrhizal plants of aeschynomene were significant only at the intermediate level of applied P. Overall, inoculated plants of the three legumes studied had greater P concentration than control plants. Maximum root colonization, expressed as either percentage or total length of colonized root of the three legumes, was attained at intermediate levels of applied P.

In Chapter VII, the effect of inoculation with selected VAM isolates on growth and nutrient uptake of Siratro and aeschynomene under natural field conditions at applied P levels of 10, 30, 60, and 120 kg ha^{-1} is

reported. At all levels of applied P and for all harvests, shoot dry weights of Siratro were greater for fungal inoculated plants than noninoculated plants. Differences between fungal inoculated and noninoculated plants were most marked at 30 to 90 kg ha^{-1} of applied P and diminished at 120 kg ha^{-1}. The effect of fungal inoculation on the shoot dry weights of aeschynomene, at all levels of applied P, was similar (but more pronounced) as that of Siratro. At the first harvest of Siratro, plants inoculated with _G. etunicatum_ had higher shoot dry weights than _G. intraradices_ plants at all levels of applied P. However, in subsequent harvests of Siratro and for aeschynomene the response of shoot dry weight to inoculation with the two VAM fungi was similar. Fungal inoculation resulted in at least a 30% savings (40 kg ha^{-1}) in the amount of P fertilizer required for maximum yield. Inoculated treatments had greater percentage of root colonized than noninoculated treatments at all levels of applied P. Percentage of root colonized by VAM fungi for the inoculated plants of the two legumes increased linearly with P additions up to 60 kg ha^{-1}. There were no differences in root colonization between _G. etunicatum_ and _G. intraradices_ at any level of applied P.

The results of these studies clearly demonstrate that inoculation with effective VAM fungi can increase the growth of legumes in soils that may have a high, but largely ineffective native VAM population than introduced VAM fungi under amended soil conditions. Furthermore, with highly mycorrhizal dependent crops such as tropical forage legumes, a mycorrhizal growth response may occur at P levels normally used in commercial pasture production.

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In August, 1984, he enrolled to the graduate program of the University of Florida, Department of Soil Science, and received the degree of Doctor of Philosophy in December, 1987. He has been married to Griselda Terrero since 1979 and they have one daughter, Michelle.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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